VOLUME 6 January 1957 NUMBER 1

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

Copyright 1957, By The American Society of Tropical Medicine and Hygiene
Made in U.S.A.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Editor-in-Chief, L. W. HACKETT
Assistant Editor, FLORENCE M. FROST
School of Public Health, University of California
Berkeley 4, California

Editor, Tropical Medicine and Hygiene News
LOUIS J. OLIVIER, National Institutes of Health
Bethesda 14, Maryland

Editorial Board

HAMILTON H. ANDERSON
C. ROBERT COATNEY, *Chairman*
HAROLD FARNSWORTH GRAY
HERBERT G. JOHNSTONE
EDWIN H. LENNETTE
DONALD B. McMULLEN
HARRY MOST
LLOYD E. ROZEBOOM

OFFICERS OF THE
American Society of Tropical Medicine and Hygiene

President
JUSTIN M. ANDREWS

President-Elect
DONALD L. AUGUSTINE

Vice-President
G. ROBERT COATNEY

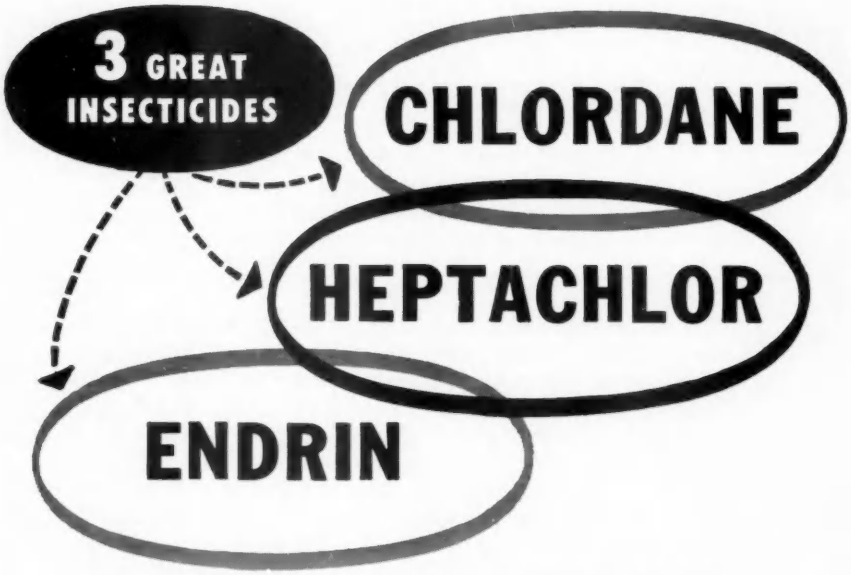
Secretary-Treasurer
ROLLA B. HILL
3575 St. Gaudens Road
Miami 33, Florida

Councilors:	*Term Expires*
ALBERT B. SABIN	1957
J. J. SAPERO	1957
MARSHALL C. BALFOUR	1958
THOMAS H. WELLER	1958
PAUL C. BEAVER	1959
WILLIAM C. REEVES	1959
JOHN M. HENDERSON	1960
J. ALLEN SCOTT	1960

Editor
L. W. HACKETT

Individuals interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care and human welfare are eligible for membership. Dues are $8 a year and include subscription to the American Journal of Tropical Medicine and Hygiene. An application blank is bound into this issue of the Journal for the use of interested individuals. Before being submitted to the Secretary, each application must be signed by two active members of the Society and a check for $8 (U.S. banks preferred) must accompany the application.

Entered as second-class matter March 16, 1921, at the Post Office at Baltimore, Maryland, under the act of March 3, 1879.
Subscription price $10.00 per volume. Single numbers, $2.00.



CONTROL THESE INSECTS

CHLORDANE: Ants, Armyworms, Blister Beetles, Boxelder Bug, Brown Dog Tick, Cabbage Maggot, Carpet Beetles, Cattle Lice, Chiggers, Cockroaches, Crickets, Cutworms, Darkling Beetles, Dog Mange, Earwigs, Fleas, Flies, Grasshoppers, Household Spiders, Japanese Beetle Larvae, Lawn Moths, Lygus Bugs, Mole Crickets, Mosquitoes, Onion Maggot, Onion Thrips, Plum Curculio, Sarcoptic Mange, Seed Corn Maggot, Sheep Ked, Silverfish, Sod Webworms, Southern Corn Rootworm, Strawberry Crown Borer, Strawberry Root Weevils, Sweet Clover Weevil, Tarnished Plant Bug, Termites, Ticks, Wasps, White Grubs, Wireworms...and many others.

HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Asiatic Garden Beetle Larvae, Black Vine Weevil, Root Maggots, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevil, Cotton Fleahopper, Cotton Thrips, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, False Wireworms, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrips, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Spittlebug, Strawberry Root Weevils, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ...and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevil, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

WRITE FOR FULL PARTICULARS

VELSICOL CHEMICAL CORPORATION

VELSICOL

General Offices and Laboratories
330 East Grand Avenue, Chicago 11, Illinois

Foreign Division
350 Fifth Avenue, New York 1, N. Y.

REPRESENTATIVES IN PRINCIPAL CITIES

(In writing to advertisers, please mention the journal—it helps.)

Abdominal tenderness

—usually confined to the lower quadrants and at times found only over the cecum—is the most frequently appearing physical manifestation of amebiasis.[1]

KOHN[2] gives a simple, quick method for identifying Endamoeba histolytica in the feces. A small amount of feces is first dispersed in saline solution. If the feces are formed and amebic cysts are likely to be present, solution 1 is used (1 cc. liquefied phenol, 0.6 cc. glacial acetic acid and 50 cc. distilled water). When feces are fluid and vegetative forms are suspected, solution 2 is substituted (0.9 cc. liquefied phenol and 50 cc. distilled water). Two or three drops of the proper reagent are placed on the slide and a loopful of the feces-saline dispersion is added; a cover-glass is applied. The solutions afford a rapid means of differentiation by changing the refractive index of the cells. When the reagent for identifying cysts is used, chromatoid bodies in the cells stand out clearly as rods, bars or short spindle-shaped bodies. Solution 2 outlines details of the nuclear structure, vacuoles and ingested material in the trophozoites.

● ***For nondysenteric colonic amebiasis*—MILIBIS®**

1 tablet 3 times a day for from 7 to 10 days is most commonly used and "has an efficiency of nearly 80 per cent."[3]

● ***For hepatic amebiasis*—ARALEN® phosphate**

2 tablets daily for from 2 to 3 weeks—"because of the toxicity of emetine and because of the efficiency of chloroquine [Aralen], chloroquine has taken the place of emetine as the drug of choice."[3]

SUPPLIED: Milibis—tablets of 0.5 Gm.
Aralen phosphate—tablets of 0.25 Gm.

● ***For complete amebiasis therapy and prophylaxis*—**
MILIBIS with ARALEN COMBINATION TABLETS
0.25 Gm. Milibis and 75 mg. Aralen phosphate.

Winthrop LABORATORIES NEW YORK 18, N. Y. • WINDSOR, ONT.

Milibis and Aralen, trademarks reg. U.S. Pat. Off., brand of glycobiarsol and chloroquine, respectively.

1. Martin, G. A., Garfinkel, B. T., Brooke, M. M., Weinstein, P. P., and Frye, W. W.: *J.A.M.A.*, 151:1055, Mar. 28, 1953.
2. Kohn, J.: *Jour. Trop. Med.*, 53:212, Nov., 1950.
3. Information Please: *GP*, 4:91, Sept., 1951.

(In writing to advertisers, please mention the journal—it helps.)

THE AMERICAN JOURNAL OF
TROPICAL MEDICINE AND HYGIENE

Vol. 6 *January 1957* *No. 1*

CONTENTS

The present status of sanitary engineering in the tropics. A review paper. JOHN M. HENDERSON 1
A dietary survey in the Santa Cruz area of Bolivia. RUTH L. HUENEMANN, HANS A. BRUCH T. AND ROBERT T. SCHOLES 21
Studies on the pathogenicity of various strains of *Entamoeba histolytica* in the rabbit. ARNE V. HUNNINEN AND HOWARD A. BOONE 32
The pharmacological basis for the prolonged antimalarial activity of pyrimethamine. CARL C. SMITH AND JEAN IHRIG 50
Electron microscope studies of Sindbis virus: a newly recognized arthropod-transmitted virus. REGINALD L. REAGAN, FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER 58
Studies on diarrheal diseases in Central America. I. Preliminary findings on cultural surveys of normal population groups in Guatemala. M. DORTHY BECK, J. ANTONIO MUÑOZ AND NEVIN S. SCRIMSHAW 62
The relapse state in typhoid fever treated with chloramphenicol. KENNETH C. WATSON 72
Ecology of ringworm fungi on commensal rats from rural premises in southwestern Georgia. WILLIAM W. SMITH, ROBERT W. MENGES AND LUCILLE K. GEORG 81
Human isosporiasis: a report of three cases with necropsy findings of one case. B. H. WEBSTER 86
The use of erythrocyte sensitizing substance in the diagnosis of leptospiroses. I. The sensitized erythrocyte agglutination test. DOROTHY E. MCCOMB, D. J. W. SMITH, DAVID L. COFFIN, ROBERT A. MACCREADY AND R. SHIHMAN CHANG 90
The use of erythrocyte sensitizing substance in the diagnosis of leptospiroses. II. The sensitized erythrocyte lysis test. R. SHIHMAN CHANG, D. J. W. SMITH, DOROTHY E. MCCOMB, C. F. SHARP AND J. I. TONGE 101
The portal pressure in hepatic fibrosis associated with bilharziasis. M. A. ARAFA, ERNEST BIBAWI AND A. RAAFAT 108
Mosquitoes associated with sylvan yellow fever near Almirante, Panama. HAROLD TRAPIDO AND PEDRO GALINDO 114
Forest mosquitoes associated with sylvan yellow fever in Nicaragua. PEDRO GALINDO AND HAROLD TRADIPO 145
The distribution of *Culex p. pipiens* and *C. p. quinquefasciatus* in North America. A. RALPH BARR 153
Canine ascariasis as a potential source of visceral larva migrans. FRANK A. EHRENFORD 166
Fly production studies in urban, suburban, and rural privies in southeastern Georgia. JOHN W. KILPATRICK AND H. F. SCHOOF 171
Studies on electrophoretic fractions of *Vipera xanthina palestinae* venom. S. GITTER, S. KOCHWA, A. DE VRIES AND M. LEFFKOWITZ 180
Books received 190
Book reviews: *Industry and Tropical Health* II. Proceedings of the Second Conference of the Industrial Council for Tropical Health; *Practical Medical Mycology*, by Keeney; *Composting*, by Gotaas; *Cryptococcosis, Torulosis or European Blastomycosis*, by Littman and Zimmerman; *Diseases of the Chest*, by Hinshaw; *Annual Epidemiological and Vital Statistics, 1953*, World Health Organization 190
Corrigendum 194

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE is issued bi-monthly, appearing in January, March, May, July, September, and November. One volume a year is issued.

Accepted articles for which immediate publication would seem important will be inserted in the next issue of the journal to go to press if the author is willing to pay the manufacturing cost. The insertion of such articles will not affect the publication of other manuscripts awaiting their turn, since the inserted articles will constitute additional pages to the volume without additional cost to the subscriber.

Manuscripts and Books for Review should be sent to Dr. L. W. Hackett, School of Public Health, University of California, Berkeley 4, California.

Correspondence concerning business matters, including advertising, should be addressed to The Williams & Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Subscription price: $10.00 per volume, United States; $10.50 Canada; $11.25 elsewhere.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be refunded to the subscriber.

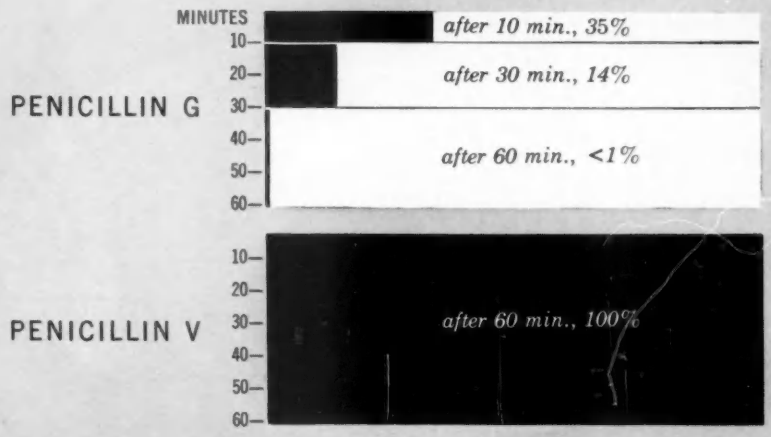
Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Change of Address: Publisher must be notified 60 days in advance. Journals undeliverable because of incorrect address will be destroyed. Duplicates can be obtained (if available) from the publisher at the regular price of single issues.

THE WILLIAMS & WILKINS COMPANY
BALTIMORE-2, MARYLAND, U.S.A.

(In writing to advertisers, please mention the journal—it helps.)

Comparison of stability of penicillin G and penicillin V in acid media



The penicillins have been subjected to a pH of 1.5 at 37°C. at the stated time intervals. The percentages shown express the residual potency.

Lilly
QUALITY / RESEARCH / INTEGRITY

The penicillin designed specifically for oral administration

V-CILLIN

(Penicillin V, Lilly)

'V-Cillin' is the only penicillin that passes through the stomach without significant loss of potency and is rapidly absorbed in the duodenum. Thus, 'V-Cillin' usually gives you a clinical dependability comparable to that of parenteral penicillin. In fact, the literature generally agrees that 'V-Cillin' can be effectively and safely used in many conditions previously treated parenterally.

Dosage: 125 to 250 mg. (200,000 to 400,000 units) t.i.d.

Supplied: Pulvules—125 and 250 mg.

Pediatric suspensions—125 and 250 mg. per 5-cc. teaspoonful

Also, 'V-Cillin-Sulfa' (Penicillin V with Triple Sulfas, Lilly), tablets and pediatric suspension

733004

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U. S. A.

(In writing to advertisers, please mention the journal—it helps.)

THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

APPLICATION FOR MEMBERSHIP

To the Membership Committee:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

(TYPE OR PRINT)

Name ..... (*Last*) ..... (*First*) ..... (*Middle*)

Mailing Address: Street ..... City .....

State ..... Country .....

Date of Birth ..... Place .....

Special Field of Interest .....

Education: Institution (Graduate and Postgraduate) Degree Date

.....

.....

.....

Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

* Proposed by:

(1) .....

(2) ..... Signature of Applicant

* Two active members must sign this application.

Return this application to Dr. Rolla B. Hill, Secretary-Treasurer, 3375 St. Gaudens Road, Miami 33, Florida

(*Check for $8.00 (U. S. banks requested) must accompany this application.*)

PEAK PERFORMANCE AGAINST INSECT PESTS

TIFA

TODD INSECTICIDAL FOG APPLICATOR

FACTORY MAINTENANCE WINTERTIME REPAIRS · OVERHAULS!

Factory mechanics check, service or recondition your TIFA machines. Get ready for next season . . . *let experts* protect your investment! For details, contact either Brooklyn or Houston plant.

OVER 12 YEARS Successful World-Wide Operation in Public Health Programs

BEST BY TEST in more than 2000 communities throughout the world. TIFA gives concentrated coverage at lowest operating and maintenance cost of any fog applicator. TIFA quickly envelops large areas, permeates smallest crevices with a true clean fog, wet or dry. Unit handles all formulations . . . solutions, emulsions or suspensions. One-man operation.

Carrying Underwriters' Listing Seal

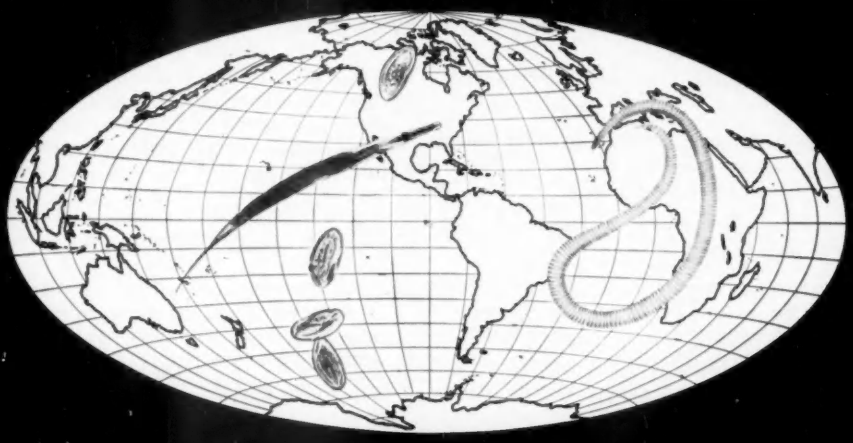
Write for illustrated folder with details and official comments

PRODUCTS DIVISION

TODD SHIPYARDS CORPORATION

HEADQUARTERS: Columbia and Halleck Sts., Brooklyn 31, N. Y.
PLANT: Green's Bayou, Houston 15, Texas

for "This Wormy World"



Pleasant tasting

'ANTEPAR'® brand

PIPERAZINE

SYRUP · TABLETS · WAFERS

Eliminate **PINWORMS IN ONE WEEK**
ROUNDWORMS IN ONE OR TWO DAYS

PALATABLE · DEPENDABLE · ECONOMICAL

'ANTEPAR' SYRUP – Piperazine Citrate, 100 mg. per cc.
'ANTEPAR' TABLETS – Piperazine Citrate, 250 or 500 mg., scored
NEW **'ANTEPAR' WAFERS** – Piperazine Phosphate, 500 mg.

Literature available on request

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, N. Y.

JOURNAL OF

PARASITOLOGY

Official Organ of American Society of Parasitologists

A medium for the publication of papers on Animal Parasites (Protozoa, Helminthes, Arthropoda).

It also publishes the program and abstracts for the annual meeting of the American Society of Parasitologists in a Supplement.

Regular numbers appear in February, April, June, August, October and December.

Subscription price, **$7.50 the year** (Foreign, $7.90). Subscriptions should be addressed to the Treasurer, Dr. Robert M. Stabler, Colorado College, Colorado Springs, Colorado.

LEON JACOBS
Chairman, Editorial Committee

JOURNAL OF PARASITOLOGY
Laboratory of Tropical Diseases
National Institutes of Health
Bethesda 14, Maryland

BEST AND TAYLOR
Physiological Basis of Medical Practice

Sixth Edition

The *U. S. Armed Forces Medical Journal* said of an earlier edition of Best and Taylor: "This book should be in the library of every physician regardless of his specialty, for the practice of medicine is founded upon a thorough knowledge of physiology." The *New York State Journal of Medicine* calls Best and Taylor a "must book for every doctor's library."

The thoroughly revised sixth edition fully merits the praise given earlier editions. If your edition is earlier than the fifth, it's time for a new one.

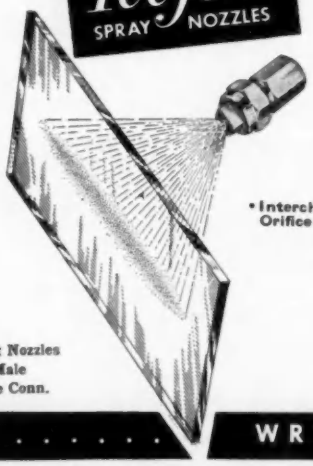
1370 pages over 600 figs. $12.00

The Williams & Wilkins Co.

Mt. Royal and Guilford Aves.
Baltimore 2, Md.

for the control of insects by SPRAYING

SPRAYING SYSTEMS
TeeJet
SPRAY NOZZLES



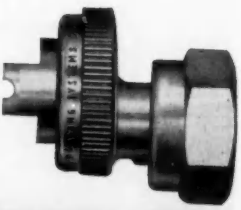
Proper atomization plus uniform distribution are the spray characteristics that make precision-built TEEJET nozzles ideal for spraying of insecticides. Full range of interchangeable orifice tips provide capacities for every type of chemical and every method of application. Complete nozzle includes built-in strainer with monel metal screen.

SPRAYING SYSTEMS CO.
3234 RANDOLPH STREET • BELLWOOD, ILLINOIS
(Suburb of Chicago)

• Interchangeable Orifice Tips

MultEEJET TIP

For use with portable sprayers. Turret head indexes to four positions to give selection of four different spray patterns. Can be used with Trigger TEEJET for handy shut-off control.



• TeeJet Nozzles Built in Male or Female Conn.

WRITE FOR CATALOG 30

(In writing to advertisers, please mention the journal—it helps.)

notes from a MICROSCOPIST'S NOTEBOOK

FAT STAINING

Techniques and solutions for the demonstration of fat in tissue have advanced markedly since Daddi's first oil soluble dye for this purpose in 1896.

Using alcohol as the solvent for the dye, early workers often dissolved out much of the fat present. Subsequently, solutions based on equal parts of 70% alcohol and acetone were used, and more recently solutions of 50% to 60% isopropanol and ethylene glycol or propylene glycol have found favor.

Advances in solutions have been paralleled by better techniques and the availability of a wider selection of fat stains which provide greater depth of color and contrast.

National has steadily expanded its line of Biological Stains and Indicators to include the principal stains in current use for demonstrating fat in tissue.

For your convenience, we list their Catalog Numbers:

#673—Sudan II	* *#629—Sudan Black B*
* *#635—Sudan III*	*#689—Oil Red O*
* *#542—Sudan IV*	*#599—Oil Blue N*
	* *#686—Nile Blue A*

*Certified by the Biological Stain Commission

1957 Silver Anniversary of the American Society of Medical Technologists

NATIONAL BIOLOGICAL STAINS and INDICATORS

PRODUCTS OF THE PHARMACEUTICAL LABORATORIES
NATIONAL ANILINE DIVISION
ALLIED CHEMICAL & DYE CORPORATION
40 RECTOR STREET, NEW YORK 6, N. Y.

(In writing to advertisers, please mention the journal—it helps.)

SMITH SPRAYERS

"For 70 Years Choice for Quality the World Over"

For Mosquito & Malaria Control

For Residual House Spraying or Larviciding Operations. Herculoy Silicon Bronze Sprayers are available in 4 gallon and smaller sizes and Armco Zinc-grip steel sprayers are also available in smaller sizes.

SEND FOR CATALOG

On our complete line of hand, continuous, compressed air, knapsack, bucket, wheelbarrow and barrel sprayers. Also hand and crank powder insecticide dusters. We are the originators of sprayers and our products are of outstanding quality in engineering, modern design and performance.

SMITH E-Z KNAPSACK SPRAYER

Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Tank solid copper or Armco zinc-grip steel. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight—20 lbs.

SMITH D.D.T. SPRAYER

The SMITH DDT Sprayer consists of a 4 gallon heavy welded galvanized corrugated steel tank, tested for hard service; equipped with brass air pump and brass air-check valve; adjustable carrying strap with snap ends, air-pressure gauge, 7-foot length of oil resisting hose; automatic shut-off control; 2-foot extension rod and all brass precision-built adjustable or fixed type nozzles. A custom built, superior sprayer. Heavy duty industrial use.

One in case.
Shipping weight—13 lbs.

SMITH No. 16D DUST KING (quart-size) DUSTER

Unexcelled in performance. Has floating plunger; does not wear out; very easy to operate. Superior in design and construction. Approved by Experiment and Extension Services.

SMITH No. 44 GIANT DUSTER

The Finest Made—Universally Approved

Unexcelled for mosquito and malaria control—when dusting is being done. Duster is precision built, well balanced, very light to carry and easy to operate. Superior in quality and performance.

One in case
Shipping weight—12 lbs.

D. B. SMITH & COMPANY, 432 MAIN ST., UTICA 2, NEW YORK, U. S. A.
ESTABLISHED 1888

THE PRESENT STATUS OF SANITARY ENGINEERING IN THE TROPICS

JOHN M. HENDERSON

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia

The term "sanitary engineering," like "preventive medicine" and others which pertain to both scientific fields of activity and professional practice, may be employed either in a strictly professional sense or in a generic sense. As a determinant of the metes and bounds of this article, "sanitary engineering" is interpreted as encompassing both the professional and the generic aspects, without attempting to draw any fine lines of distinction between the problems, interests and activities of professional engineers on the one hand and non-engineers on the other. In the large, difficult and complex task of controlling the tropical environment in the interest of man's health and welfare, there is obvious room for contributions by many arts and skills. Appraisal of these problems and of their indicated solutions can only be confused by efforts to subdivide them into respective professional packages.

There is one important exception. Sanitation is thought of as a basic part *of* or *within* public health. To an increasing degree, the sanitary engineer is also concerned with all matters which encompass public health (except under its most extended definition), and which variously are basic *to* and basic *within* public health, and also follow the attainment of a state of health and economic well-being. In essence, this is the total environmental control concept.

Now a brief note as to the term "tropics." We are employing it in its popular meaning, rather than in its strict geographical definition. Accordingly, by "tropics" is meant all areas which conform to the classical definition, together with many other very extensive contiguous areas. In terms of climate the total area may be broadly categorized into two major groups: a) the true tropics which are characterized by warm, humid, nearly uniform temperatures throughout the year, and b) the hot, parched areas of the world outside of the United States. Located frequently in the temperate zone, the parched lands often are popularly considered tropical because of elevated temperatures resulting primarily from lack of rainfall, or poorly distributed rainfall during the year. Interspersed within the confines of these two broad areas are smaller, isolated ones which, by virtue of elevation, have the climatic attributes of the temperate zone. They include not only mountainous areas but also tablelands with or without mountains. These too are generally considered tropical from a public health and tropical medicine viewpoint because they possess other characteristics associated with the tropical areas around them.

Excluding Australia and the United States, the "tropics" thus include the great expanse of territory comprising all of South Asia, almost all of Africa, the Caribbean Islands and most of the Pacific Islands, and all of Central America

and most of South America, where a billion people live, residing for the most part in mud or thatched huts. As has so often been said before, the tropics possess a large proportion of the world's population, the great bulk of the world's communicable disease, much of the world's resources of critical raw materials and generally a state of relative economic underdevelopment.

The population of most of this area lives in a basically rural environment. In great contrast to highly industrialized countries, 80 to 90 per cent of the people depend on the land for their livelihood and live in community settlements, usually villages. Sheer lack of per capita agricultural production prevents the economy from supporting a higher urban proportion. It is only in those countries which are but marginally underdeveloped, such as some in Latin America, that the supply of domestic and imported food encourages somewhat higher proportions of urban populations.

Under this setting, improvement in food supply, and hence rural area development, quite properly should precede further urbanization and the development of heavy industry, even though all require ultimate attention. But a more productive agriculture will depend not only on improved methods but on the health of the farming population. In consequence, we are concerned with such elementary things as the village well and rural excreta disposal rather than the more elaborate systems developed for urban societies.

THE DISEASE PROBLEM

With some localized exceptions, the two principal disease groups of the tropics are considered by most to be what might be termed the malarias and the filth-borne diseases. Both of these are environmental disease groups. The filth-borne disease group in particular usually forms a complex with malnutrition. These problems are closely followed by tuberculosis, which achieves this rank not on account of sheer incidence but because of its long drawn out disabling chronicity and high fatality rate. Although it possesses certain direct environmental aspects, tuberculosis can more appropriately be classed as a generalized socioeconomic problem.

Since malaria has been selected as the subject of another special review article, further consideration of tropical sanitary engineering from a disease standpoint will be concentrated on the filth-borne diseases.

The principal filth-borne diseases are considered to be cholera, shigellosis, the salmonelloses including typhoid and paratyphoid, amebiasis, and the helminthiases, particularly schistosomiasis and ancylostomiasis. In terms of sheer incidence, certain other specific worm infections of filth-borne origin such as ascariasis outrank this list, but are less important due to lack of evidence of serious physiologic and pathologic damage. As Sabin (1956) has so aptly pointed out: "We must clearly differentiate between incidence of infection and incidence of disease."

One might speculate as to how it is possible for anyone to glibly classify diseases by their importance in areas where morbidity and mortality statistics are conspicuous by their absence, or at least by their incompleteness, and where

medical diagnostic services are extremely scanty. As for rates, accurate population censuses have never been made in many of the areas, except in a few principal urban centers.

In the rural areas of the tropics where the great bulk of the people tend to live, three levels of morbidity record keeping are generally encountered: areas where no records at all are available, those where fever cases and deaths are distinguished from non-fever episodes, and those where a more detailed classification is attempted but which is so incomplete and inaccurate that its superiority over the former is questionable, at least during endemic periods.

In spite of these shortcomings, medical intelligence has devised ways and means of appraising disease incidence over broad areas. In relation to the scope of this article, one of the most useful tools is the calculation of life expectancy at birth which, for the tropics as a whole, is reported to approximate 30 years. By comparison, this figure for the United States is 70 years (1954).

It is generally recognized that extremely short life spans such as in the order of 30 years are primarily the result of high infant mortality rates and that the great infant killers are the diarrhea and enteritis (primarily shigellosis)-nutrition complex and malaria. Where malaria is absent or where it exists at moderate levels, the leading cause of infant death by process of elimination becomes the filth-borne infections. Where hyperendemic malaria is present, responsibility must be shared by both groups of diseases since the filth-borne diseases are an important problem throughout the tropics.

Of far greater value is the method of appraising public health problems in selected local areas and applying these results to broader areas with similar ecologic characteristics. The preferred procedure is by comprehensive survey as was done by Weir *et al.* (1952), but special limited surveys, clinic records and the experience of practicing physicians have some value, albeit variable, in evaluating the problem.

As an index of the importance of filth-borne diseases in rural Egypt, Weir's study of five villages yielded the following results: Ninety-five per cent of the population had cyst-confirmed stools positive for *Entamoeba histolytica*. Data were not presented in this article on shigellosis and other infections, but in spite of the high amebiasis rate it was stated that the majority of the extremely common diarrheas and dysenteries were considered to be the result of bacterial infection. Ninety-five per cent of the people also were found positive for *Schistosoma hematobium* or *S. mansoni*. Based on serological results corroborated in part by case histories, the annual attack rate of typhoid and paratyphoid fever was estimated at 1,800 per 100,000. Indicative of early massive typhoid-paratyphoid infection was a positive serological rate of 40 per cent among children 0 to 4 years of age. The observed typhoid carrier rate was 12.5 per cent but the actual rate was believed to be higher due to unsatisfactory collecting technique. Although the area was served by a health center, 65 per cent of the infant deaths and about 25 per cent of the births were not reported. The infant mortality rate approximated 200 per thousand live births.

The cultural and physical environmental circumstances which make possible

the maintenance of disease transmission at these levels in Egyptian villages have been well described by Scott (1939) and Chandler (1954). They include not only lack of potable water supply, sanitary excreta disposal devices and swarms of house flies which in numbers and moisture-seeking behavior must be experienced to be appreciated, but cultural practices which seem purposely designed to encourage maximal enteric disease transmission. The floor of the customary animal room in the mud hut dwelling which serves as a joint collecting place for animal and human excreta, the frequent handling of these mixed materials for various purposes, lack of handwashing, and a way of life in close contact with dirt floors add greatly to the dwelling place problem. To this should be added the use of uncomposted and semicomposted night soil in the cultivation of food crops. Indicative of these practices was a village *Ascaris* infection rate of as high as 87 per cent and of over 60 per cent in the 1–4 age group, as reported in Weir's study. In contrast, the hookworm infection rate was only 4 per cent and did not rise above 7 per cent in any age-sex segment. On this basis, it is doubtful if any frank hookworm disease existed.

While it is doubtful that filth-borne disease hazards are greater in any other underdeveloped areas than in the typical Egyptian village, Egyptian practices are far from unique. Replicas of all or most elements of the Egyptian environmental complex can be found at least in the greater part of the Arabic world. As for India, Engler (1956), using the round number technique, indicates the environmental status by citing an annual toll of 2 million lives and 50 million cases of illness from filth-borne and water-borne infections. Comparable figures for malaria in India until very recently were 2 million deaths and 100 million cases.

Nor is the importance of the filth-borne diseases limited to the tropical areas of the Eastern Hemisphere. According to Molina and Puffer (1955), "gastritis, enteritis, etc." were the leading causes of death in 1952 in Brazil, Colombia, Costa Rica, the Dominican Republic, El Salvador, Guatemala and Mexico and the second leading cause in Chile and Panama. Their study did not include all countries in the tropical Americas.

It is on such varied building blocks as these that the importance of the filth-borne disease group may be appraised.

Yet there are also extensive regions where environmental conditions, though still primitive and hazardous by Western standards, are free of one or more of the hazards faced by the Egyptian villager. Schistosomiasis, for example, is a multi-regional problem associated largely with certain types of irrigation, but it is far from being tropicopolitan. Religious customs in some areas encourage practices of frequent bathing and washing among accepted elements of society which may far exceed "Western" standards, although they may otherwise encourage enteric disease transmission by the drinking of polluted water during bathing. Although extensive, the use of night soil outside of Europe is mainly confined to areas with Oriental or Arabic cultures and it is not universal in these.

While it is regrettable that objective epidemiologic surveys of the type carried out in the Egyptian village study have not been more extensive in the tropics, even such studies provide only a partial answer to the health status of the areas

specifically surveyed. When 95 per cent of a permanent population is chronically infected with at least two potentially lethal micro-organisms, virtually all of the working members of the community are infected as well as the infant and the elderly. The fact that the community is able to survive is in itself proof that the classical host-parasite principle is actively functioning. Adjustment of parasite to host is exemplified by benign strains of *E. histolytica*, while in the case of observed "inapparent typhoid," one might postulate that immunity is the paramount factor. As another index of adjustment, 76 per cent of the population had a hemoglobin level of between 90 per cent and 100 per cent in spite of the massive schistosome and ascaris infection rates and the poor dietary status. This index was depressed by low values in infants and young children and was materially more favorable among other age groups. In any event, once the critical barrier of the early years of life has been successfully crossed, the criterion of disease from a socioeconomic standpoint becomes not laboratory-detectable infection (which tends to overstate) or even disabling illness (which understates). Rather, it is in large measure the high prevalence of energy sapping, life shortening non-disabling infection. This phenomenon is not unique for the filth-borne diseases. One is reminded of the African villages having estimated malaria transmission rates of up to 50 infections per capita per year, and superficially healthy adult populations. Epidemiological surveys have yet to develop a practical standardized technique for measuring the boundary line between infection *per se* and economically crippling disease, and may never do so. Yet, as with malaria, the major cost of hyperendemic enteric disease to society is apt to be not the cost of funerals or animal sacrifices or even time lost from work, but the 365 days spent each year in semi-productive work due to disease. Such costs at present can be rationally determined in rural areas only by eliminating disease first and then measuring the gain in crop production.

Although figures for some of the enteric infections in round numbers are available for many countries, in the face of the foregoing there is little point in attempting to tally the hundreds of millions of cases of those filth-borne infections which have been specifically cited as ranking problems. It seems preferable to state that the incidence of filth-borne diseases is high in all of the underdeveloped tropical areas of the world, although the specific infections may vary widely in prevalence from place to place, and that in the seasoned professional judgment of many observers, it is a ranking public health problem and perhaps *the* ranking problem. Those who are more statistically inclined are referred to Stoll's (1947) detailed enumeration of over 2 billion worm infections alone. However, this figure must be heavily discounted when one attempts to separate significant disease from infection.

THE SOLUTION

It is a simple matter to spell out the theoretical solution to the problem.

Full control of filth-borne infections requires: 1) a safe, adequate water supply under pressure within each dwelling; 2) elimination of house flies or of access by them to human and animal feces; 3) elimination of fecal pollution from snail-

inhabiting surface waters, or elimination of the snail vector; 4) the production, processing, preparation, storage, and consumption of food under sanitary conditions; 5) observance of hygienic practices of a high standard within the home, especially in the care of infants and young children, and a dwelling and neighborhood environment which encourage and make possible such practices; 6) absence of soil pollution responsible for helminthic infections; 7) the essential absence of domestic rodents, vermin, and domestic animals from dwellings; 8) favorable natural factors and agricultural practices which make possible the existence of a stable non-nomadic community in the interest of developing hygienic and sanitary improvements; 9) a type of culture and religion which encourages, or at least permits the attainment of a favorable hygienic and sanitary status; 10) an educational status which contains the teaching and understanding of disease causation and makes possible attainment of a favorable economic status; and 11) an economic status which makes possible the financing of the indicated environmental improvements.

The difficulty of achieving these 11 objectives is so apparent that it is obvious "full control" cannot be extensively realized at any early date. Three main courses of action accordingly present themselves. The first is to do nothing. In most of the countries involved, this course is politically infeasible, or at least undesirable, on the part of the governments in power. It is not implied that the general public is dedicated to the cause of filth-borne disease control, but rather that a substantial fraction desires better living conditions, achievable under the foregoing 11 point program and other measures.

The second course, which is not focused on filth-borne disease control or even concerned with public health as the primary objective, involves recognition that the betterment of urban and rural living conditions is not only complex but that fundamental improvement is apt to be long drawn out. The village development programs which have been initiated by the governments of a number of countries are perhaps the best example of the modified application of this philosophy.

The third course is to attempt to convert the idealistic objectives of complete filth-borne disease control into a more realistic step-by-step or "useful unit" type of program. Such programs may be a part of village development programs, or may be independent in whole or in part.

Village water supplies. In rural areas, the improvement of village water supplies commands first attention in any step-by-step program. Although not as basic to the control of filth-borne disease as the safe disposal of human excreta, village water supply development is indicated as a first step for the following reasons.

a) Rural populations in most underdeveloped areas are concentrated in villages, nomads excepted. Isolated individual dwelling places are rare.

One to three wells can often serve an entire village, whereas other filth-borne disease control facilities and measures tend to be on an individual family basis. In consequence, village water supply development generally is economical.

b) Water is a basic physiological necessity for animal and plant life, including man, and there is often keen awareness of its importance on the part of villagers. Community action and support can be more readily obtained for water supplies

than for almost any other improvement. The convenience motive can often be exploited.

c) The provision of a safe drinking water supply in the absence of other measures generally reduces the incidence of several of the filth-borne diseases, especially the typhoid fevers.

d) Acceptance of an improved water supply by the community involves fewer cultural and occupational changes than other filth-borne disease control measures.

While the merits of village water supply development were not entirely over-

FIG. 1. E 22002. An ancient deep dug well at an oasis in the Arabian desert. Water is lifted in four goatskin bags by ropes and pulleys.

FIG. 2. E 22001. Animal motive power for Figure 1

looked by many state and national governments in earlier years, attention has been focused on them in many countries through the various technical assistance programs of the United States. These have included many Latin American countries, beginning in the 1940s under the aegis of the Institute of Inter-American Affairs, and more recently in other parts of the world under the various Point IV programs. In Africa and Asia, for example, village water supply develop-

FIG. 3. E 19096 (top left). Getting water from a dry riverbed in India
FIG. 4. E 21999 (top right). Indian villagers digging a well
FIG. 5. E 22004 (bottom). One of the better types of older dug wells in India

ment programs have been instituted in Afghanistan, Ethiopia, Thailand, India, Nepal, Pakistan, Viet Nam, Lebanon, Egypt, Jordan and the Philippines. At the same time, the need for urban water supply development has not been overlooked and the services of American engineers in this field have been supplied to a more limited extent in these and other countries. The most ambitious of the village water supply programs is in India, where the 5-year goal is to serve more than 550,000 villages with 1,650,000 wells (Engler, 1956). In addition, India's vast program of irrigation development has not been restricted to surface water supplies, but has involved the relatively extensive construction of large capacity tube wells. To some extent this program provides drinking water supplies as an adjunct to irrigation water supply.

Typically, village water supply development in Asia and Africa comprises providing a single, or a very few, wells per village, but no distribution system. The pattern in some of the Latin American programs has been more varied and has included limited distribution systems which serve strategically located public hydrants, public baths and laundries, and simple types of slow sand filters to treat surface water from irrigation canals.

Although village water supply development of the Eastern Hemisphere type is the simplest and most economical of the filth-borne disease measures, it is not without its difficulties. Aside from the very important factors of logistics, financing, personnel training and organizational development, improved village water supplies, although popular, are not always automatically accepted. Ingrained taste preferences and social practices may, at times, result in continued use of the older, polluted source even though it may be less conveniently located. Sociologically, acceptance of water supply development is slightly more difficult than DDT residual spraying for malaria control (where only passive action may be required), in that a degree of positive action is required on the part of the people. On the other hand, acceptance is vastly simpler than most other indicated prevailing methods of filth-borne disease control which involve major changes in cultural and/or hygienic practices. In a nonmechanical society, maintenance of even hand pumps may be difficult to obtain.

A far simpler, more rapidly progressing operation than the more comprehensive village development program, village water supply development in most cases can be carried out successfully as a separate entity. However, existence of a village development program in the community can be expected to expedite and further the success of a village water supply project and may at times be essential.

Aside from hydrologic conditions which may dictate design or even contraindicate the use of wells, the questions of sanitary standards, the relative adequacy and quality of existing water supplies, and the extent of local self-help desired have important bearing on where to carry out the work and what type of well to construct. Some of these factors are competitive, requiring the establishment of priorities or making compromises.

Existing sources of water supply among village and town groups are of many types. Among them are springs, streams, wells—shallow and deep; dug, drilled

and bored; protected and unprotected; irrigation canals; natural and artificial pools, such as "tanks"; and imported water which may be distributed by goatskin bag or kerosene tin. Of these, the "tank" and some irrigation canals provide classic examples of disease transmission potentialities. The community "tank" typically is an excavated ground pool from a fraction of an acre to several acres in size in which water is collected from the immediate surrounding area during the rainy season. It is generally located adjacent to the village. It may be used for purposes of community water supply, ablution after defecation, bathing, laundering, and as a wallow for domestic animals; offering optimal opportunity for the interchange of a community's own filth-borne organisms. The irrigation canal is distinguished because irrigation is most commonly found in arid and semi-arid climates where these channels are often the only source of water for any purpose; because populations tend to be dense in irrigated areas and villages and towns tend to be located along canal banks and because the canal serves the functions of the "tank" but on an inter-community basis. Many natural streams play a comparable role in community water use.

Offering somewhat lesser opportunities for enteric disease transmission are unprotected springs, inadequately or improperly curbed existing wells, staircase wells and dug wells which are fully protected from surface and subsurface pollution but from which water is drawn by lowering drinking water vessels which may be contaminated to some extent. In a country such as India, many of these wells are centuries old. At the top of the scale in sanitary safety are deep bored wells with hand pumps and tubing, and possibly water obtained from temporary holes dug in the sand-gravel beds of rivers during extended seasonal droughts.

It is apparent that under such varied conditions perplexing decisions must be made in the planning and design of some national village well-development programs.

For example, should new well construction be designed to meet optimal sanitary standards; i.e., eliminate any possibility of water-borne disease transmission? If so, installation of pumps is a *sine qua non* except in the case of flowing artesian wells. Tube wells, either drilled or bored, are favored over any other type. The chain of consequences includes: a) problems of pump maintenance in a mechanically primitive village society, b) substantial-to-great increase in necessity for materials, equipment, personal services and funds from non-local sources, with proportionate reduction in local self-help.

The popularity and acceptability of new wells among village populations may be considered to be directly related to the following:

a) is the present supply adequate in quantity?

b) is the new supply more convenient to use?

c) are there any strongly established cultural or aesthetic preferences favoring the old supply over the new?

The provision of new wells in "tank" villages appears to offer better prospects for success of village well development than all other situations except where water is in dire shortage. Granted that strong cultural preferences in favor of the old supply may exist in individual cases, that use of the "tank" for non-

drinking water purposes will continue, and that some may drink water while bathing. Offsetting merits are:

a) hydrologic conditions in "tank"-village areas tend to be favorable for construction of inexpensive dug wells;

b) wells generally can be placed in the center of the villages while the "tank" generally is on the outskirts;

c) the communicable disease control case for providing wells in "tank" villages is outstanding.

The principle of self-help and the desirability of making it an integral part of any program of health betterment have been expounded so often, there is little point in stating the case again. May we point out, however, that self-help is a relative term, both in the minds of individuals and agencies, and in its functional applicability. To the social anthropologist, self-help usually means a personal contribution by the individual or family receiving benefit, or at the very least by the immediate community of which he is a part. This is in considerable contrast to some American technical assistance programs where participation may be 10 per cent from United States sources and 90 per cent from local sources, but in which 100 per cent of the "local" support is from the national and state governments of the recipient country. Such circumstances are dictated by the nature of the project and by tax practices. In countries where all property taxes and virtually all other taxes except those for business licenses are collected by the national government on behalf of all governmental units, local governments are seldom handed more than the "pig's grunt."

Regardless of the merits or demerits of such systems of taxation, it is also true that some projects inherently require public participation rather than private, and even state or national rather than local participation. Perhaps the best example are the current national malaria eradication programs which are being carried on or proposed so energetically in many countries. Probably no measure could advance prospects for the development of anopheline resistance prior to the realization of malaria eradication more certainly than for responsibility for the application of DDT residual spray to be placed on the individual householder! Moreover, success in national malaria eradication is dependent in part on eliminating gametocyte carriers from the last community, irrespective of local interest. Other projects in a public category include municipal systems for water and sewage distribution, collection and treatment. Village well development is in a somewhat different category. Almost from time immemorial, villagers by concerted voluntary action have dug and bored wells and have curbed them, using primitive construction methods and materials.

Except for the provision of outside technical supervision in the interest of sanitary quality, village well development on a 100 per cent self-help basis is feasible, especially in village development areas. It is only in the interest of achieving high standards of sanitary quality, including provision of pumps, and expediting rates of progress, that outside support beyond technical supervision is indicated where dug and bored wells are hydrologically feasible.

Village excreta disposal devices. In approaching this subject, brief consideration

must be given to prevailing village excreta disposal practices. In certain Oriental countries where land is intensively cultivated, such as Japan and large areas of China, human excreta is considered an important organic resource for crops and stock raising. Conservation methods include: a) the collection of night soil (undigested fecal matter and urine) from urban areas by commercial operators, and within the family by individual village householders, for application on croplands; b) the anaerobic composting of human excreta in combination with other animal and vegetable wastes and the subsequent application of the digested material on cropland; and c) the feeding of human excrement to the family hog, as in north China. However, as may be expected due to wide variations in culture and land cultivation, practices over the Asian land mass lack uniformity. While the use of night soil and compost is by no means limited to Japan and China, there are many countries where neither is conserved in village husbandry. Other practices include community defecation grounds around ablution water sources, use of flat roof tops with parapet walls by children and women and of shallow open pits by men, in villages and towns in feudal Arabia, and shoreline defecation within the daily tidal zone in certain coastal Arabic areas. Egyptian villagers widely use the animal room from where the material is eventually placed on top of the ground in a mixed compost pile (mainly anaerobic), or latrine dump. The only universal practice appears to be defecation at site by workers in the fields.

As is apparent from this array of practices, opportunities for the transmission of filth-borne infection by skin-soil and skin-water contact, by the direct ingestion of infected water and raw food, by the fly route and by exposure routes within the dwelling place, are many albeit widely variable.

During much of the twentieth century, the method of source control of this varied problem generally advocated by public health authorities has been covered burial, as first prescribed by Mosaic law. The usual procedures advocated are the provision of sanitary pit privies and bored hole latrines and a number of extensive projects of this type are underway today, both with and without extranational technical aid.

Since the adoption and consistent use of these devices often involves substantial change in personal habits and at times runs counter to established mores, it might be considered that improvement in excreta disposal practices can succeed only as a phase of long range comprehensive village development undertakings. That is, the sanitary disposal of human excrement can succeed only as a sequel to the basic economic and educational uplifting of the community, supplemented by applied public health education. Considering that the indicated objectives include consistent use of the device by all ambulant members of the family, and adequate maintenance, in addition to installation, we believe few would take issue in principle with this concept.

Under the circumstances it is surprising to note the occasional popularity of sanitary privies and latrines in some villages where they have been introduced with limited advance preparation. In certain Moslem areas, for example, the lack of preexisting alternative facilities providing privacy are reported to have

spurred the demand for privies by women (Perine, personal communication). At another location, the enthusiastic acceptance of bored-hole latrines was found to be due to the novelty of the earth-auger used in boring the holes. The villagers were "willing to pay almost any price within their means for the privilege of experimenting with this wonderful toy for a few hours" (Adams, Foster and Taylor, 1955). We mention these instances, not in disparagement, but to illustrate that new excreta disposal customs may be promptly adopted when convenience, privacy, novelty or some other more immediate motivation than the prevention of disease accrues. At the same time one wonders if these motivations are strong enough to insure use by all members of the family and to what extent the facility will be maintained.

Quite apart from the sociologic aspects of sanitary excreta disposal devices is the question of their inherent value as a method of filth-borne disease control. When adequately and consistently used, the practical effectiveness of pit privies and bored hole latrines for the control of soil pollution by hookworms was clearly confirmed many years ago. Under suitable hydrologic, soil and usage conditions, their effectiveness in the reduction of surface water pollution and the avoidance of ground water pollution is equally clear. However, the effectiveness of pit privies in preventing house fly production in the pit contents and especially in preventing fly feeding is far less clear. This is of particular interest in view of the apparent importance of house flies in transmitting filth-borne bacterial infections in areas of high endemicity.

As one who had a hand in earlier years in the construction and subsequent operating experience of some tens of thousands of so-called sanitary pit privies in the southern United States, it became quickly apparent to me that few remained fly-tight very long after construction. Later observations in other countries have confirmed this experience. This seemed of no great consequence at first because one was told that house flies flew from darkness into light and furthermore, didn't produce to any extent in human excrement.

Since there is common agreement that house flies do feed on and produce in human excrement to some extent when the material is exposed on the surface of the ground, the issues are those of the degree of preference for human excrement and whether house fly behavior patterns are interrupted by sanitary pit privies and bored-hole latrines under practical operating conditions. It is probable that these issues can never be resolved by generalized conclusions in view of the many variables. These include the versatility of this ubiquitous insect, the fact that there are many house fly species, subspecies and biotypes, that climatology and other ecological factors in its distribution range vary widely and that the environments and physical condition of the privies themselves likewise vary. Thus, the house fly's behavior differs greatly with time and place, and even the casual American traveler is keenly aware that its behavior in the middle East bears little resemblance to that of those back home.

As regards physical design features, it is rational to assume that the bored hole latrine in principle is a far more effective device in preventing house fly production and feeding than is the sanitary pit privy and is to be preferred wherever

soil, hydrologic and usage conditions are favorable to its use. But due to its lower effective capacity at times, the bored hole latrine can be less satisfactory than the pit privy under conditions favoring the latter. Observations of pit privies indicate that the extent of house fly feeding and production is more directly influenced by ease of access into the pit, i.e., the condition of the pit cover, and by the moisture content of the pit, than by depth and illumination. Recent observations in this country have tended to be centered on the phenomenom of the vastly expanded production in privy pits which have been treated with dieldrin.[1] Although the following observation is subject to some qualification when applied to non-dieldrin situations, Kilpatrick and Bogue (1956) classified house fly-producing privies as to darkness and depth of pit. The series included dark, deep (over 6 feet below the surface) sanitary pit privies and they concluded that depth of pit or amount of light had little effect on fly breeding potential. Heavy production of *M. domestica* took place in deep, dark privy pits as well as those which were more shallow, had more light, or both.

Of principal public health interest in the house fly complex are *Musca nebulo*, *Musca domestica cuthbertsoni*, *Musca domestica domestica*, *Musca domestica vicina* and *Musca sorbens*, although there are many other species and subspecies. The evidence indicates an ascending preference for human excrement as a production medium in competition with other media by the last three species. *M. sorbens* in Egypt appears to have an almost exclusive preference for human excrement (Sabrowski, 1952; Peffly, 1953), and a strong predilection for man's company. The dominant, but less pronounced preference of *M. d. vicina* in Egypt was well documented by Holway *et al.* (1951) with data obtained from over 2,200 observation points. Schoof and Siverly (1954) found *M. d. domestica* in 45 per cent of a total of 270 pit privies examined at Phoenix, Arizona, during different seasons. Seventy-four per cent of those examined in the summer yielded *M. domestica* larvae. The pits ranged from 2 to 6 feet in depth and the authors noted that *M. domestica* infested human feces under conditions which were considered of low light intensity. Dieldrin was not considered a complicating factor.

Yet most vector control workers will agree that, while *M. domestica* production in privies is widespread in this country, its quantitative significance usually is secondary to that from other production media and that certain non-house fly species produce in larger numbers in privies, dieldrin situations excepted.

It is presumed that house-fly feeding on human excrement within and outside of privies in poorly sanitated areas throughout the world is a usual practice irrespective of where the flies produce.

Indicative of the relative importance of house flies in transmitting filth-borne infection in poorly sanitated areas, and of the imperfection of sanitary pit privies due to the combined effects of lack of usage and inherent characteristics of the device, was the experience of Weir *et al.* (1952) in the Egyptian village study. In two villages where effective fly control by chemical methods was temporarily achieved, infant mortality rates were reduced very sharply, suddenly increasing when chemical control failed. At the time fly control was in effect, water supply

[1] Less marked increases have been observed for privies treated with chlordan and BHC.

and sanitary latrine services were only partially installed. Subsequent complete coverage of these services without fly control did not reduce infant mortality rates by comparison with control villages nor change the fly status. No conclusions were drawn as to the possible reduction in enteric fever morbidity which may have resulted as a result of water and latrine installation. This demonstration involved the use of bored-hole latrines in the presence of a high ground water table.

An added disadvantage of pit privies, and possibly bored-hole latrines, is the heavy production of the *Culex fatigans* (*quinque fasciatus*) which frequently takes place in pits which extend into the ground water table. At best, these mosquitoes constitute nuisance problems, while in some areas they are the primary vector of filariasis.

On balance, it would appear that effective improvements in excreta disposal sanitation by present methods in most underdeveloped Asiatic areas at least, are contingent on comprehensive socioeconomic village development (second course program), and should not be adopted as a spearhead activity (third course program). We take this position from a standpoint of immediate communicable disease control results to be expected in proportion to effort expanded. A possible exception would be high hookworm areas where the partial use of sanitary excreta disposal devices might be expected to reduce worm burdens to more tolerable levels. The counter position might be that the advancement of sanitation consciousness is an integral part of the basic educational process and that care in the disposal of human excreta is the first step in sanitation. Under this concept, the sanitary pit privy and the bored hole latrine become primarily educational tools and only secondarily a method of immediate, direct filth-borne disease reduction.

Alternatives. 1. The importance of house flies in the transmission of filth-borne diseases, and of acute ophthalmitis in some areas might lead some to advocate fly control in preference to excreta disposal sanitation. However, there is at present no satisfactory chemical attack against heavy house fly production. Sanitation measures required to avoid severe fly problems are even more comprehensive and sociologically involved than excreta disposal sanitation. Selective control, such as against *M. sorbens*, is directly dependent on excreta disposal sanitation.

2. Many observers have commented on the greater appeal of agriculturally motivated cultural change over that which is public health motivated. Adams *et al.* (1955) point out that the two near-universal reasons why villagers are willing to change their ways are visible economic advantage and prestige and honor. In a village society, agricultural improvements tie directly in with both of these compelling motivations. As a result "it is far easier to bring about improvements in agriculture and animal husbandry than in sanitation and other health measures." In India, Leavell (personal communication) reports "that under the conditions as they exist here people in the villages are much more ready to accept new agricultural practices than they are to adopt sanitary latrines," and that "there are very numerous examples o` adoption of new agricultural practices."

This and much other evidence suggests that a fundamental change in approach toward excreta disposal practices may be needed for village areas if progress is to be expedited. The development and introduction of more satisfactory simple composting practices involving the conservation of human and other animal excreta along with vegetable wastes is the main indicated procedure. Recent research (U. of Cal. 1953 and others) has demonstrated the feasibility of a high-rate natural thermophilic composting process which generates temperatures high enough to kill the most resistant pathogenic organisms and to substantially control fly production. Sufficient oxygen to provide requisite aerobic conditions and the removal of waste gases can be accomplished by occasionally turning over the compost pile. Whether this process can be feasibly developed for adoption by villagers, or whether the slower anaerobic method of earth covered burial is indicated has not been satisfactorily established. In either event, the indicated approach to this and other village sanitation developments is along practical rather than perfectionistic lines. In some areas, for example, an ascariasis-ridden population might be tolerated if a main transmission route of the dysenteries and typhoid could be severed. Nor for that matter do we consider that the early objective of any village water supply program should be the attainment of an essentially coliform-free water.

3. A compelling motive which at times may be omitted from the sociologists' list of felt needs is the instinct of man and other animals to obtain relief from suffering. Hence, provision of clinical treatment often has been considered an essential entering wedge for the introduction of any preventive medicine measures, including sanitary excreta disposal devices. This is quite apart from the essential direct role that clinical medicine may play in the control or eradication of certain diseases under certain circumstances. Without denying this essential position in some types of public health programs, or the vital contribution made by clinical practitioners in paving the way for, and carrying out, preventive medicine programs, relief from suffering per se does not confer on those benefited a burning desire for personal hygiene and sanitation. There have been, in consequence, abundant examples where preoccupation with medical care and the heavy drain on public funds have served to perpetuate disease problems. The effective combination of clinical and preventive medicine requires the local services of a completely developed physician and usually a public health team. The relatively heavy and highly selective staffing involved in areas where even clinical practitioners are conspicuous by their absence, makes such programs limited to special demonstration areas.

4. Last, but not to be overlooked, are potentialities for the improvement of sanitary excreta disposal devices without appreciable increase in price. The greatest room for improvement is toward encouragement of greater use and improved dependable flytightness. We have noted, for example, mention of a concrete slab of the Asiatic squatting plate type, provided with a hand flushed water seal trap to prevent the passage of flies, mosquitoes and odors. The reported cost of this latrine, less superstructure is Rs. 20 to 25 (U.S.$4 to $5).

Village development programs. Brief mention of village development programs is

indicated in view of their relationship to rural filth-borne disease control activities. As program elements, village development projects have as their ultimate objective the comprehensive socioeconomic uplifting of a community, usually in a series of many steps. Although the concept is not new, village development programs in underdeveloped countries have expanded rapidly in number and

FIG. 6. E 22367. A village house in India with fuel cakes in foreground. (Draft animals are essential, but they compete with man for food supply and their manure is used for fuel, rather than to replenish the land.)

FIG. 7. E 22366. Primitive wooden plows in an Indian village

magnitude during the postwar period. They seek to achieve more surely and permanently by democratic processes, economic improvement in agrarian societies comparable to that being sought by forced action in rural China, and to realize social gains not obtainable by dictatorial processes. The largest program is in India, but national programs are being carried on in other Asiatic countries, Africa, South America, the West Indies, the Philippines and possibly elsewhere. In addition to many articles in popular publications, such programs have been rapidly reviewed by three teams (Adams *et al.*; Carley *et al.*; Gibson *et al.*, all 1955). More gradual evaluations by governmental and private reviewers are underway.

Due to diversities of culture and governmental structures and attitudes in this broad area, village development programs necessarily vary widely in nearly all elements and in accomplishments.

However, the sociologic aim which characterizes most of them is the development by the community of "felt need" and "self help." Where community initiative (felt need) does not develop spontaneously, it is aroused and stimulated by social science techniques. Community self-help may vary from a nominal contribution to 100 per cent of the total except for the cost of technical advisory services. Depending on felt need and governmental plans, the first step may comprise development of one or more of the following: literacy in the vernacular, agriculture, animal husbandry, communications and transportation, home care housing, cottage industries, water supply, other specific sanitation measures, and public health education.

The objective of the community development program in India is to ultimately serve 558,000 villages. Using this country as an example, a representative plan of organization is to provide the services of a multi-purpose village level worker (VLW) for each of about 5 villages for grass roots stimulation. Technicians drawn from government departments are located in the "Block," comprising 100 villages. These include such persons as a block leader and an agriculturist, veterinarian, public health specialist, cooperative specialist, and a social education organizer. Higher echelon units are supervisory and consultative in function. As is apparent from the magnitude of the program and the diversity of function of the multi-purpose VLW, care in recruitment selection, and the quality and adequacy of personnel training are of great importance. It is in the area of training that Point IV technical assistance in village development is mainly concentrated.

An alternative plan, used in the Philippines, relies on the direct service of specialists in education, agriculture, public works, social welfare and public health without provision of any multipurpose workers or coordinating personnel.

To illustrate some of the complexities and perplexities of village development, we quote below without comment, excerpts from two reports. Both deal with India. The first is by an American public health administrator and reflects his current observations at the time. The second is by an Indian public health engineer and refers to the past.

"The concept of felt need has perhaps been overemphasized. . . . Existing needs, which we can recognize, need to be defined and their fulfillment demon-

strated before they become felt needs of the Indian villager. . . . Self help is a concept that appeals strongly and holds the promise of permanence . . . . It should, however, not be applied in such a way as to exclude the creation of demand through demonstration. . . . A widespread demonstration in village sanitation paid for as a part of the project, should be an integral part of the community development project, in order to develop felt need and show the way to self help. . . . The concept of multipurpose workers requires constant review . . . the total capacity of the workers remains severely limited. . . . The multipurpose worker gives official sanction to Indian folk medicine and preserves certain more sanitary folklore." Hyde, H. V. (1952), in a mimeographed report on a trip to India.

"Many village uplift centers were opened in the past both by official and non-official agencies. These well-intentioned attempts have however borne no tangible fruit. Conditions have reverted to the original as soon as outside influence was withdrawn." Singh, Purtej (1953).

TOTAL ENVIRONMENTAL CONTROL

In common with the trend toward a broadened outlook in their respective fields by some other professions, the sanitary engineering profession has become increasingly concerned with the total picture of the environmental balance sheet in an area as it affects mankind. In effect this might be considered an element of the positive health concept, and so it is, yet it has its own distinct characteristics. Applicable to both highly developed and underdeveloped areas, it has even greater applicability to the latter because of the relative lack of development of available resources. Neither impractical nor visionary, "environmental engineering" as Logan (1954) terms it, represents the concept that the part should be viewed only in relation to the whole and that there should be integration of planning and execution between all engineering specialties and other sciences and professions. Dividing environmental control practice into the six elements of water, climate, earth, sunlight, biological factors (other than man) and man himself, he discusses the planning and application aspects of environmental control in each of these six fields. Thus, water as an environmental health resource and liability is given as much consideration from the standpoints of irrigation, flood control, navigation, hydroelectric production, land reclamation and industrial requirements as with respect to quality and quantity for potable water supply. This resources planning engineering concept has been expressed along similar lines in recent years by many other sanitary engineers and expert committee groups. Rather than being a novel concept, to many it marks a return to and an extension of traditional civil-engineering thinking with its strong emphasis on economic planning and the development of basic resources.

Even in the highly limited field of preventive medicine (as viewed from the Olympus of positive health), the endpoint of the civil-engineering contribution is not readily determinable, as demonstrated by Euclidean process: Epidemic cholera is associated in India with famine. Famines commonly have resulted from regional crop failures and lack of transportation to bring in food from other

parts of the country. Hence, highway and railroad development prevents epidemic cholera. Q.E.D.

REFERENCES

ADAMS, H. S., FOSTER, G. M., AND TAYLOR, P. S., Team I, 1955. *Report on community development programs in India, Pakistan and the Philippines*. International Cooperation Administration, ppbd. 88 pp.

CARLEY, VERNA A., AND STARCH, ELMER A., Team II, 1955. *Report on community development programs in Jamaica, Puerto Rico, Bolivia and Peru*. International Cooperation Administration, Washington, D. C., 76 pp.

CHANDLER, A. C., 1954. A comparison of helminthic and protozoan infections in two Egyptian villages two years after the installation of sanitary improvements in one of them, *Am. J. Trop. Med. & Hyg.* **3:** 59–73.

ENGLER, HERSHEL, 1956. Engineering job opportunities in world health, *J. San. Eng. Div., Proc. of the ASCE* **82**(SA 3): 1012-1–8.

GIBSON, WILLIAM C., MASTERS, HUGH B., AND WITTE, ERNEST F., Team III, Dec. 1955. *Report on community development programs in India, Iran, Egypt and Gold Coast*. International Cooperation Administration, Washington, D. C., 101 pp.

HOLWAY, R. T., MITCHELL, W. A., AND SALAH, ABDEL AZIZ, 1951. Studies on the seasonal prevalence and dispersal of the Egyptian housefly. Part II. The larvae and their breeding areas, *Ann. Entomol. Soc. America* **44:** 489–510.

KILPATRICK, JOHN W., AND BOGUE, MALEN D., 1956. Adult fly production from garbage can sites and privy pits in the lower Rio Grande Valley, *Am. J. Trop. Med. & Hyg.* **5:** 331–339.

LOGAN, JOHN A., AND BURDEN, ROBERT P., 1954. The public health significance of environmental control by engineering means, *Bull. of Hyg.* **29:** 221–231.

MOLINA, G., AND PUFFER, R., 1955. Report of health conditions in the Americas, *P. H. Reports* **70:** 943–950.

PEFFLY, R. L., 1953. A summary of recent studies on house flies in Egypt, *J. Egyptian Pub. Hlth. Ass.* **28:** 55–74.

PEFFLY, R. L., 1953. The relative importance of different fly-breeding materials in an Egyptian village, *J. Egyptian Pub. Hlth. Ass.* **28:** 167–80.

SABIN, A. B., 1956. Medical and hygienic problems in the development of the tropics—the responsibility of the U.S.A., *Trop. Med. & Hyg. News* **5:** 4.

SABROSKY, CURTIS W., 1952. House flies in Egypt, *Am. J. Trop. Med. & Hyg.* **1:** 333–336.

SCHOOF, H. F., AND SIVERLY, R. E., 1954. Privies as a source of fly production in an urban area, *Am. J. Trop. Med. & Hyg.* **3:** 930–935.

SCOTT, J. A., 1939. Observations on infection with the common roundworm, *Ascaris lumbricoides*, in Egypt, *Am. J. Hyg.* **30:** Sect. D, 83–116.

SINGH, PURTEJ, 1953. Present state of sanitary conditions in rural areas and small communities in India, WHO Working Paper/Env. San./34. Mimeo. 6 pp.

STOLL, NORMAN R., 1947. This wormy world, *J. of Parasitol.* **33:** 1–18.

University of California Sanitary Engineering Research Project, 1953. Reclamation of municipal refuse by composting, *Technical Bulletin No. 9, Series 37*, 89 pp.

WEIR, J. M., WASIF, I. M., FARAG RIZK, H., ATTIA, S. M., AND ABDEL KADER, M., 1952. An evaluation of health and sanitation in Egyptian villages, *J. Egyptian Pub. Hlth. Ass.* **27:** 56–114.

A DIETARY SURVEY IN THE SANTA CRUZ AREA OF BOLIVIA[1]

RUTH L. HUENEMANN[2], HANS A. BRUCH T. AND ROBERT T. SCHOLES

Department of Nutrition, Harvard School of Public Health, Boston, and Service Cooperativo Interamericano de Salud Publica, La Paz, Bolivia

As part of a general survey made by the Health Servicio of Bolivia (SCISP)[3] to determine the health needs of the Santa Cruz area (Scholes *et al.*, to be published, 1956), a dietary study was conducted in the town of Montero and its immediate vicinity in the Santa Cruz Department in August, 1955. Its prime purpose, like that of the entire health survey, was to establish a basis for future program planning. Since a program, to be effective, must relate itself acceptably to existing interests and practices, an attempt was made to obtain data that would give a picture of present ways of eating and living and an indication of people's feelings about them, as well as a quantitative measure of food intake. Health, Agriculture, and Education Servicios collaborated in the dietary study, for all were rightly concerned with the food and nutrition problems of the country.

Household dietaries and the food intake of post-weaning children under four years of age were studied, the latter because this age group has been shown to be particularly vulnerable to deficiency disease (Colonial Office, 1954; Autret and Behar, 1954; Brock and Autret, 1952).

Exploration of possible relationships between levels of nutrient intake and physical findings will be discussed in a future publication after sufficient year-round dietary data have become available to justify such a procedure.

DESCRIPTION OF MONTERO AREA

The town of Montero has a population of about 2,300, mostly mestizos. It is located in an area of large agricultural plantations. The climate of the region is subtropical, with an average maximum temperature range of about 76–88°F., and an average minimum of about 58–70°F. Extremes range from 102°F. to 32°F. Average annual rainfall is approximately 43 inches, about one-half of which falls during the three-month period of November to January. There is a pronounced dry season, and serious droughts may occur in the June–September period. Frequent high winds, especially in the dry season, are characteristic of the area. The soil is predominantly light-textured, loamy sand with a wide range of drainage conditions, and the natural vegetation is largely low jungle type. Better types of this soil are suitable for the production of rice, corn, cotton, citrus fruits, bananas, peanuts, yuca and improved livestock pasture.

[1] Supported in part by a contract between the Institute of InterAmerican Affairs of the International Cooperation Administration and Harvard University and by general funds of the Harvard School of Public Health.

[2] Present address: School of Public Health, University of California, Berkeley.

[3] Servicio Cooperative Interamericano de Salud Publica, cooperative public health service between the governments of the United States and Bolivia, administered through the Institute of Interamerican Affairs, International Cooperation Administration.

The area in general is underdeveloped and living standards are low. Health facilities are inadequate and felt health needs are few. A sanitary census of Montero in March, 1954, (unpublished data) disclosed that of the total living quarters, 56 per cent were without latrines, 2.6 per cent had satisfactory facilities for disposal of excrement, and the remainder had inadequate disposal facilities. A large scale intestinal parasitological survey done in May–July, 1954, (Scholes *et al.*, to be published, 1956) disclosed an infection rate of 90 per cent; 85 per cent were infected with hookworm, the majority heavily so; 54 per cent had roundworm infections. A large range of specific infections was noted, and 69 per cent had multiple (2–5) infections.

PROCEDURE

Sampling method. For the urban area, a two per cent random sample of households was drawn using a map[4] as a frame and applying the systematic sampling technique by choosing one house among the first fifty at random and from this base selecting every fiftieth house for the sample, following a serpentine line throughout the map. The sample for the rural area was selected in two stages: first, a sample of three farms out of fifteen was chosen using the systematic technique described above, and then a list of households on these farms was made and a random sample of ten per cent was chosen. Thus a sample of twelve urban and eight rural households was obtained. Of the eight rural households, two were those of landowners and the remaining six were those of employees of the two landowners. Of these six, two received their food already cooked from the landowner and four were responsible for securing and preparing their own food. The twelve Montero households appeared to be representative of the various socio-economic levels of the town.

In twelve of the twenty households the food intake of a post-weaning child between the ages of one and four years was also studied.

Gathering data. Field staff for the survey consisted of a statistician, a nutrition consultant, and seven interviewers recruited from the local health department and from the three Servicios. The seven included a social worker, a sanitarian, a secretary, a chauffeur, a home agent, and two auxiliary health workers (public health nurses' aides).

Interviewers were given one day's instruction in general interviewing techniques and in the specific aims and methods of this study, as well as an opportunity for the less experienced to observe and work with the most experienced interviewers of the staff before they gathered data by themselves. They visited the homes in the survey once or twice each day for a week to obtain a record of the household food intake and, in homes where there was a weaned child under four years of age, a record of his intake as well. In order to have data as quantitatively accurate as possible, workers carried scales and weighed household food and portions for the selected post-weaning children whenever possible. Throughout the week some information regarding types of homes, living conditions, educational level of the people, and their attitude toward their present food practices was also recorded.

[4] Map was made by the personnel of the Rural Mobile Unit of SCISP in Montero.

Analysis of data. At the end of the week food intakes were summarized and average daily consumption determined for households and post-weaning children. Intakes were calculated in nutrient terms by using values from a Peruvian food table (Collazos *et al.*, 1953) and from the United States Department of Agriculture Handbook No. 8 (U. S. Department of Agriculture, 1950). Results were then compared with the recommended dietary allowances of the National Research Council (National Academy of Sciences—National Research Council, 1953), adapted to a mean environmental temperature of 20°C. and to the weight and age of the individuals included in the survey.

Reporting of data. At the close of the week preliminary findings of the survey were discussed at a meeting of leading citizens of the area. The group consisted of the personnel of the Montero Health Unit, the agricultural agent, a representative of the school, the priest from the local church, the chairman of the women's club, and interested homemakers. The suggestions for improving dietary practices that grew out of this discussion are included later in this report.

As soon as compilation of the data was complete, findings were reported to the three Servicios involved and immediately used in work-shops for their personnel and in program planning.

FINDINGS

People and their homes. The population surveyed was essentially a young one, both in the town and rural areas. Of the 142 persons in the survey households, only 30 were 40 or more years old. Average household size was 7.1 persons. About 63 per cent of the people aged five and over were able to read, even though 40 per cent of these had not attended school. Literacy was higher in the urban than in the rural population, for of those 5 years of age and over in the town, 77 per cent could read, while in the rural area only 39 per cent of this age group were literate. Only three people in the rural area and none in the town had attended a secondary school. Montero had only an elementary school.

Of the twelve urban households, five owned their own homes, two rented theirs, two lived in loaned houses, and three did not specify who owned their houses. The two farm owners lived in their own houses, while the six farm-worker families lived in houses loaned them by the farm owners.

Homes and living conditions were simple. Houses were generally built of mud or adobe, whitewashed in some cases, with roofs of thatch or tile and either earthen or brick floors. Eleven of the twenty houses consisted of but one room in addition to the kitchen. Of the twenty kitchens, four were in the open air, four were partially walled with a roof, eleven were indoor kitchens without windows, and one had a window. None had chimneys. Nine had brick stoves of standing height, while eleven had low stoves built with the fire chamber directly on the floor. Nine had no ovens. Kitchen utensils consisted of aluminum and earthenware pots, tin cans, wooden spoons, gourds, and a few iron frying pans. Wood was the only fuel used. Water was obtained from private or neighborhood wells or springs, except in three urban households that bought the water they used.

Household food intake. Table 1 lists the foods appearing in the dietaries of

TABLE 1

Average daily food intake of persons in 20 households during survey week, August, 1955

Food	Urban Area Total of 88 persons in 12 households			Rural area—Farm Owners (1) Total of 19 persons in 2 households			Rural Area—Farm-Workers Total of 35 persons in 6 households		
	No. of households using specified food	No. of persons in households consuming specified food	Average daily intake per person	No. of households using specified food	No. of persons in households consuming specified food	Average daily intake per person	No. of households using specified food	No. of persons in households consuming specified food	Average daily intake per person
			gm.			gm.			gm
Fresh beef	12	88	57	2	19	110	5	29	22
Dried beef	7	42	25	1	12	13	3	15	6
Pork	7	52	15	2	19	19	4	29	8
Chicken	4	35	12	2	19	25	1	11	5
Liver	3	22	7	0	0	0	0	0	0
Canned sardines	1	3	20	0	0	0	0	0	0
Fish, fresh	0	0	0	0	0	0	1	4	3
Fish, dried	1	3	16	0	0	0	0	0	0
Eggs, number of	10	78	0.24	2	19	0.7	2	15	.07
Milk	6	43	39	2	19	65	0	0	0
Cheese	1	3	5	2	19	8	0	0	0
Butter	0	0	0	1	7	9	0	0	0
Lard or fat	12	88	17	2	19	25	6	35	4
Rice (uncooked wt.)	12	88	106	2	19	173	6	35	116
Rice bread	8	60	24	0	0	0	2	15	10
Wheat bread	12	88	142	2	19	66	4	26	28
Wheat flour	4	35	7	1	7	22	1	4	1
Corn	6	41	8	0	0	0	1	5	5
Corn bread	4	33	23	1	12	65	1	11	13
Macaroni	8	66	7	2	19	18	0	0	0
Yuca	9	69	62	2	19	60	6	35	129
Potatoes	10	78	18	2	19	41	0	0	0
"Papalisa" (a tuber)	4	28	11	1	12	16	0	0	0
Tomatoes	4	34	13	1	12	4	0	0	0
Lettuce	3	21	15	2	19	9	0	0	0
Onions	12	88	6	2	19	5	4	26	1
Cabbage	2	15	5	2	19	6	0	0	0
Carrots	3	30	5	1	7	15	0	0	0
Artichoke	2	24	2	0	0	0	0	0	0
Palm sprouts	0	0	0	1	12	4	0	0	0
Bananas	5	42	21	1	7	17	3	19	34
Grape fruit	2	23	96	0	0	0	0	0	0
Lime	1	12	20	0	0	0	1	11	29
Oranges	0	0	0	1	7	62	0	0	0
Orange marmalade	1	11	44	0	0	0	0	0	0
"Chicha" (a non-fermented corn beverage)	6	47	64	0	0	0	1	2	271
Sugar	12	88	72	2	19	72	6	35	31
Sugar cane honey	2	23	13	0	0	0	2	11	13
Alcohol	0	0	0	0	0	0	1	4	44

(1) Data for 6 days only.

households surveyed, with a calculated daily average per person. Coffee, consumed in all households, was not recorded. Rice, yuca, bread, and sugar will be seen to constitute the major part of the diet in all households. Breads were made of refined, non-enriched flours, either wheat, rice, or corn. All households had some meat and eggs, although the amount for rural farm workers averaged less than thirty grams per person per day in most households, while the two farm owners' households had about 150 grams daily per person, and urban intakes averaged 60 grams or more per person per day. The fat intake of farm workers' households was likewise low. In farm owners' homes eggs were consumed at the rate of almost one daily per person, while farm worker households averaged only about one-half egg per person per week and urban households about two weekly per person. Rural farm workers had no milk, while one-half of the urban dwellers had about 40 grams per day and farm owners about 65 grams per person daily. Farm workers' households consumed no vegetable except onions and yuca, but some of the urban and the farm owner households did consume small amounts of potatoes and other vegetables. Farm workers' households consumed proportionately more yuca and less bread than either of the other two groups. Typical menus for the three groups were:

	Farm owner household	Farm worker household	Urban household
Breakfast	Bread with butter or cheese Coffee with milk and sugar	Yuca or bananas Dried beef occasionally Coffee with sugar	Bread Coffee with sugar
Dinner	Cabbage soup (rice, cabbage, carrots) Eggs Lettuce with oil dressing Yuca	Beef and rice soup Yuca	Beef and rice soup (sometimes with potato and vegetables) Yuca
Supper	Casserole of rice, beef, and onions Coffee with sugar	Rice with small amount of lard or meat Yuca or bread Coffee with sugar or cane syrup	Bread Coffee with sugar

Conversation with families indicated that at certain seasons of the year meat intake tended to be generally lower and citrus fruit consumption higher than at the time of this survey. Future studies will be needed to substantiate this impression.

Home food production did not play a significant role in supplying families' food. Six of the twenty households reported producing yuca; five reported rice; three, eggs; two, tomatoes; and one each, milk, pork, lettuce, carrots, bananas, grapefruit, onions, and sugar cane.

When families were asked how they felt about their food, only one family head expressed satisfaction with the present food supply; fifteen said that they would like but could not afford to buy milk, fresh meat, fish, vegetables and fruit; and four said they had difficulty affording milk, meat, fruit, and oatmeal.

TABLE 2

Average weekly food intake of 12 weaned children, aged 1-4 years, during survey week, August, 1955

Food	Urban Area (Total of 6 children)		Rural Area Farm-Owner (1) (1 child)	Rural Area Farm-Workers (1) (Total of 5 children)	
	No. of children consuming specified food	Average daily food intake	Average daily food intake	No. of children consuming specified food	Average daily food intake
		gm.	gm.		gm.
Fresh beef	6	33	34	5	32
Dried beef	2	22	0	1	2
Pork	4	8	0	3	9
Chicken	3	5	16	1	6
Fish	0	0	0	1	4
Liver	1	3	0	0	0
Eggs, number of	2	0.1	0.5	1	0.16
Milk	3	37	337	0	0
Lard or fat	6	12	3	5	4
Rice	6	60	66	5	127
Wheat bread	6	72	47	5	26
Rice bread	2	9	0	2	9
Corn bread	0	0	37	0	0
Wheat flour	3	4	0	0	0
Macaroni	3	9	19	0	0
Yuca	5	17	0	4	151
Potatoes	4	19	21	1	77
Corn	3	6	0	1	19
Onions	6	4	2	2	6
Lettuce	0	0	2	0	0
Carrots	1	4	0	0	0
Tomatoes	1	6	0	0	0
Bananas	4	7	0	1	27
Lime	2	14	0	1	27
Grape fruit	2	35	0	0	0
Sugar	6	27	19	4	15
Sugar cane honey	2	7	0	2	7
Orange marmalade	2	16	0	0	0
"Chicha" (sweetened corn beverage)	2	70	0	0	0

(1) Data for 6 days only.

Children's food intake. That foods consumed by the twelve post-weaning children under four years of age in the study were virtually the same as those eaten by their families is indicated in Table 2. Again, rice, yuca, bread, and sugar formed the main portion of the dietary. Only four children had any milk, ten had fruit or vegetables, four had eggs, and all twelve had meat, fowl, or fish.

Nutritive value of household dietaries. Table 3 shows the average nutritive value per person per day of household dietaries and a comparison with the mean recommended dietary allowance calculated on the basis of individual ages and

TABLE 3

Average nutritive value per person per day of 20 one-week household dietaries and comparison with recommended dietary allowances

	Calories	Protein gm.	Calcium mg.	Iron mg.	Vitamin A I.U.	Thiamine mg.	Riboflavin mg.	Vitamin C mg.
12 urban households								
Average daily intake per person	1731	52	79	7	1824	0.47	0.56	40
Standard deviation	±528	±17	±88	±5.5	±2878	±0.21	±0.24	±27
Average recommended daily dietary allowances	2113	56	925	10.8	4302	1	1.40	70
Intake as percentage of recommended dietary allowances	82	93	19	65	42	47	40	57
6 households of farm-workers								
Average daily intake per person	1320	26	143	4	314	0.22	0.20	87
Standard deviation	±657	±15	±73	±2.4	±258	±0.75	±0.09	±69
Average recommended daily dietary allowances	1936	53	911	10	4172	1	1.28	65
Intake as percentage of recommended dietary allowances	68	49	16	43	8	22	16	134
2 households of farm owners								
Average daily intake per person	2400	78	349	9	2507	0.94	0.85	70
Standard deviation	±615	±13	±116	±0.9	±12	±0.48	±0.18	±28
Average recommended daily dietary allowances	2114	61	1026	10	3919	1	1.50	65
Intake as percentage of recommended dietary allowances	114	128	34	91	64	92	57	109

weight. The outstanding dietary lack, as judged by this standard, in all three groups—urban residents, farm workers, and farm owners—was calcium. To a lesser degree, riboflavin was also low, as was vitamin A. Except for these three nutrients, the average intake of farm owners' households almost met or exceeded the recommended allowances. Farm workers' diets, on the other hand, were low in all nutrients except vitamin C. The nutrient intake of urban residents, while higher in all but vitamins C and A than the average for all rural households, was still low compared to the recommended allowances.

Distribution of nutrient intakes among urban, farm-worker, and farm-owner households in relation to their own allowances is indicated in Table 4. That mean values, of themselves, did not in all instances give a true impression of actual intakes measured in terms of individual household requirements is illustrated by the finding that 9 of the 12 urban households had less than half of their recommended allowances of vitamin C, despite an average intake of 57 per cent. High intakes of iron and protein on the part of a few households likewise brought up the mean without benefitting other households. Of the six farm-worker house-

TABLE 4

Number of urban, farm-worker, and farm-owner households meeting specified proportions of their recommended dietary allowances

	Total No. of Households	Number of households meeting specified proportions of recommended dietary allowances							
		Calories	Protein	Calcium	Iron	Vitamin A	Thiamine	Riboflavin	Vitamin C
Urban households	12								
Proportion of nutrient allowances:									
Less than ¼		—	—	8	—	8	—	1	4
¼ to ½		1	—	4	4	—	8	8	5
½ to ¾		4	3	—	5	2	3	3	—
¾ to recommended allowances		5	6	—	2	—	1	—	2
More than recommended allowances		2	3	—	1	2	—	—	1
Farm-worker households	6								
Proportion of nutrient allowances:									
Less than ¼		—	1	5	1	5	5	5	1
¼ to ½		2	3	1	3	1	1	1	—
½ to ¾		2	1	—	1	—	—	—	1
¾ to recommended allowances		2	1	—	1	—	—	—	1
More than recommended allowances		—	—	—	—	—	—	—	3
Farm-owner households	2								
Proportion of nutrient allowances:									
Less than ¼		—	—	1	—	1	—	—	—
¼ to ½		—	—	1	—	—	1	1	—
½ to ¾		—	—	—	—	—	—	—	1
¾ to recommended allowances		1	1	—	1	—	—	1	—
More than recommended allowances		1	1	—	1	1	1	—	1

holds, three had less than their recommended allowance of vitamin C, even though the mean intake was 134 per cent. The two farm-owner households differed widely in their intakes of vitamin A, thiamine, and ascorbic acid. Thus mean values in many instances tended to give a more favorable impression than warranted by individual household intakes.

Nutritive values of children's diets. In comparison to recommended allowances, children's diets tended to be lower in calories and nutrients than those of families. Table 5 shows that except for vitamin C, calories and protein, average intakes of all nutrients were well under 50 per cent of the recommended amounts. As indicated in Table 6, over 90 per cent of the children failed to obtain as much as half of their recommended allowances of calcium, vitamin A, and riboflavin.

TABLE 5

Average daily nutritive value of one-weeks' food intake of 12 weaned children aged 1–4 years and comparison with recommended dietary allowances

	Calories	Protein gm.	Calcium mg.	Iron mg.	Vitamin A I.U.	Thiamine mg.	Riboflavin mg.	Vitamin C mg.
Average daily intake per person	811	24	122	3	365	0.20	0.30	35
Standard deviation	±202	±10	±89	±1.2	±765	±0.08	±0.19	±40
Average recommended daily dietary allowances	1120	38	900	7	1867	0.60	0.90	35
Intake as percentage of recommended dietary allowances	72	63	14	42.8	20	33	33	100

TABLE 6

Number and percentage of children meeting specified proportion of their recommended dietary allowances

Nutrients	Total	Less Than One Quarter		From One Quarter to One Half		From One Half to Three Quarters		From Three Quarters to Recommended Allowances		More Than the Recommended Allowances	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Calories	12	—	—	—	—	9	75	1	8	2	17
Protein	12	—	—	4	33	5	42	2	17	1	8
Calcium	12	11	92	1	8	—	—	—	—	—	—
Iron	12	—	—	10	83	1	8	1	8	—	—
Vitamin A	12	10	83	1	8	—	—	—	—	1	8
Thiamine	12	3	25	5	42	4	33	—	—	—	—
Riboflavin	12	7	58	4	33	—	—	1	8	—	—
Vitamin C	12	3	25	5	42	1	8	—	—	3	25

Just as in the case of the household dietaries, vitamin C distribution was particularly uneven, for even though the average intake equalled 100 per cent of the mean recommendation, 67 per cent of the children failed to receive half of their recommended allowance.

DISCUSSION OF FINDINGS AND THEIR APPLICATION

While the findings reported here must be considered preliminary until checked by further surveys at other seasons of the year, there seems to be little doubt that nutrient intakes as judged by the recommended allowances of the National Research Council will be found to be extremely low at all times. Preliminary study of physical findings (to be published later), as well as the high degree of parasite infection reported here, support the view that dietary improvement is definitely indicated. The three Servicios that cooperated in this survey are, as previously stated, ready and willing to encourage a program toward this end.

The regularity with which all households ate three meals a day would seem to indicate that meal-planning is not haphazard. This fact, along with the discontent expressed by families in regard to their present food intakes, would seem to

show at least a degree of readiness for a program designed to improve food practices.

It would seem that many nutrient lacks could be alleviated by a program of home food production. Even in the town there seemed to be sufficient space and man-power available for such a program, and agriculture experts had tested its feasibility. The cultural pattern of the people did not include home food production activities, however, and patient understanding will be required on the part of agency personnel attempting to initiate such. Nutritionally speaking, a gardening program would be highly desirable, for green and yellow vegetables are especially rich sources of carotene; dark green leaves are high in iron content as well; vegetables also supply vitamin C, in which many diets were low; beans and legumes of all kinds, including peanuts, supply thiamine, riboflavin, iron and protein. Raising of fowl and small animals, such as guinea pigs and rabbits, would likewise improve the nutritional quality of the diet, especially in protein, iron, thiamine, and riboflavin. Although none of these foods is completely foreign to present food consumption patterns, people would need to be taught how to incorporate larger amounts of them into the diet.

With equal patience, understanding, and skill, other dietary changes need to be initiated. The eating of liver and kidney, for example, should be encouraged, not only for protein and iron, but, in the case of liver, also for vitamin A.

Very low calcium intakes are to be expected in a diet low in milk. Legumes, molasses, certain greens, yuca leaves, and quinoa (both cereal and leaves) might be used in larger quantities for their calcium content. Until such time as more adequate quantities of milk can be produced, perhaps families could be encouraged to divert some of the money now spent for sugar to the purchase of dried milk, available in the shops in Montero. Coffee could perhaps be drunk with milk and less sugar. The increased calcium intake and decreased sugar consumption might well reduce the incidence of dental caries. Use of molasses as a sweetening agent rather than sugar is to be encouraged because of its calcium and iron content and its lower cost.

The lack of whole grain cereals in the diet is reflected by the low thiamine intake. Oatmeal is available, but is expensive in comparison with rice and noodles. Careful budgetary planning and a lower purchase of sugar might, however, enable some families to use more of it. It is, apparently, a cereal that is well accepted. Since rural families polish their own rice, it might be possible to teach them to use some of it in less polished form. While brown rice might not meet with acceptance when served as a separate dish, it might be well liked in soups. Peanuts might also help the thiamine intake, as well as the beans and legumes previously mentioned. Pork, an outstanding source of thiamine, is well-liked but expensive. In a long-range program, however, increased hog production should be considered.

The use of more yuca in place of white bread is to be encouraged, mainly to increase the vitamin C content of the diet and also because yuca is less expensive than bread and more easily prepared with the limited cooking facilities available.

Since the post-weaning child eats family fare and no foods especially prepared for him, one obvious way to improve his diet is to improve that of the entire family. Meanwhile, families in Montero should be encouraged to obtain UNICEF dried skim milk distributed by the Maryknoll Fathers.

Other suggestions will occur to the educator as she works with the people in the improvement of their own diets. Families themselves will contribute suggestions when the results of this survey are reported back to them.

SUMMARY

A seven-day dietary survey of a random sample of twenty households and twelve post-weaning children in the Santa Cruz area of Bolivia showed generally low intake of calcium, riboflavin, thiamine and vitamin A. Calories and the remaining nutrients for which calculations were made, i.e., protein, iron, and ascorbic acid, were also low in some cases. Diets of farm labor households tended to be lower in nutrient content than those of farm-owner and urban households. Diets of the post-weaning children, ranging in age from one to four years, compared less favorably with recommended allowances than did those of households. Rice, yuca, refined bread, coffee, sugar, and varying amounts of meat constituted the major portion of all diets. A few families ate vegetables and fruits in addition, and some used very small amounts of milk. A program of home food production and possible changes in food preparation and buying practices are suggested as ways of improving dietaries.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the help of Dr. Cassal and his staff of the Mobile Health Unit at Montero, of the Agriculture Servicio (SAI), of the Education Servicio (SCIDE), and especially the technical assistance of the following persons from these agencies who served as interviewers: Melania Antelo, Mario Ardaya, Gloria Bloch, Porfidia Céspedes, Adan Gómez, Victoria Hurtado, and Leonella Rea.

REFERENCES

Autret, M., and Behar, M., 1954. Sindrome policarencial infantil (kwashiorkor) and its prevention in Central America, *FAO Nutritional Studies* No. 13.

Brock, J. F., and Autret, M., 1952. Kwashiorkor in Africa, *FAO Nutritional Studies* No. 8.

Collazos, Carlos, Ch., White, Philip L., White, Hilda S., Vinas, Eduardo T., Alvistur, Enrique J., Urquieta, Renan A., Vasquez, Juan, Diaz, Cesar T., Quiroz, Alfonso M., Roca, Amalia N., and Hegsted, Mark D., 1953. La composicion de los alimentos peruanos, *Anales de la Facultad de Medicina*, No. 2.

Colonial Office, 1954. *Malnutrition in African mothers, infants and young children.* Report of Second Inter-African (C.C.T.A.) Conference on Nutrition, Gambia, 1952. H.M. Stationery Office, London.

National Academy of Sciences—National Research Council, 1953. Recommended Dietary Allowances (Revised 1953), National Research Council, Washington, D. C.

U. S. Department of Agriculture, 1950. *Composition of foods—raw, processed, prepared.* Agriculture Handbook No. 8, U. S. Department of Agriculture, Washington, D. C.

STUDIES ON THE PATHOGENICITY OF VARIOUS STRAINS OF *ENTAMOEBA HISTOLYTICA* IN THE RABBIT[1]

ARNE V. HUNNINEN AND HOWARD A. BOONE

Division of Preventive Medicine, and Division of Medicine, University of Tennessee, College of Medicine, Memphis, Tennessee

Several species of animals (especially kittens, rats, and guinea pigs) have been used by investigators to determine the susceptibility of various host species to infection with strains of *Entamoeba histolytica*. Tobie (1949), using only a single strain of this parasite, was highly successful in producing marked infection in the rabbit. Until the present study, however, the rabbit has not been used for comparing numerous strains of *E. histolytica*.

The purpose of the present study is to compare the pathogenicity, in the white rabbit, of 37 human strains[2] of *E. histolytica* and to determine the relationship between the ability of the parasite to invade the tissues of the rabbit's digestive tract, and the presence of symptoms or pathological changes in patients from whom the strains were isolated. Information was also collected on the size of the amebae, the percentage of the rabbits which developed lesions, the distribution of the lesions along the digestive tract, the weight changes in infected rabbits, the pathological changes produced, and the size of the trophozoites in cultures compared with their size in the lesions of the rabbit.

MATERIALS AND METHODS

White rabbits, 8 to 12 weeks old, were used in the following experiments. In the early tests the rabbits were placed on a corn-oats-bread diet similar to that used by Westphal (1941) and by Tobie (1949). This diet proved unpalatable to the rabbits so it was modified by using oats-Purina rabbit pellets-cracked corn in the proportion of 3:2:1. This diet was used throughout the rest of the experiments.

The various strains of *E. histolytica* used in the present study were obtained from food handlers in Memphis, Tennessee, and also from hospital patients[3]. These strains were isolated from the feces by culture in Nelson's egg yolk alcoholic extract medium. After 48 to 72 hours of incubation, amebae from the positive tubes of each strain were separately pooled and counted by using the Howard mold counting chamber. The specimens were then divided for injection into rabbits. In the preliminary experiments 50,000 to 2,000,000 trophozoites were used per injection but since we found no correlation between the number injected and the number of lesions produced, we established approximately 200,000 as the standard injection per rabbit, sometimes less and sometimes

[1] Supported in part by a Contract with the Department of the Navy, Office of Naval Research, Washington, D. C.

[2] The term "strain" as used here indicates a difference in source of the parasites. Differences in pathogenicity or other characteristics are not necessarily implied.

[3] We are indebted to L. S. Suter, Veterans Administration Medical Teaching Group Hospital, Memphis, Tennessee, for some of the strains used in this study.

more. We also tried the L.E.R. (Locke's egg rice) medium for growing the amebae, but in every instance better results were obtained with Nelson's medium. Similar results have been reported by Laird *et al.* (1949) when comparing Nelson's medium with three others.

The rabbits were anesthetized with nembutal given intravenously. A small incision was made in the right lower quadrant of the abdomen and, after locating the cecum with forceps, the amebae were injected directly into the cecum with a 5 cc. syringe and a 19 gauge needle. The muscles were sutured and the skin was closed with Michel clips. Sterile precautions were followed in the operations.

Autopsies were performed usually between the 14th and the 20th day after infection. The number, size, and location of the ulcers were recorded. The weight of each rabbit was taken at the time of infection and at autopsy. For microscopic studies the tissues were fixed in 10 per cent formalin immediately at autopsy. Sections were stained with hematoxylin and eosin. The size of the amebae was determined by measuring the trophozoites when they were in the temporarily rounded form.

Cultures of the feces of 40 non-inoculated control rabbits were made to determine whether they harbored *Entamoeba*. All of them proved to be negative.

Below is a key used to indicate the degree of infection found in the rabbits shown in Tables 3 and 4 and in Graph 1.

Groups	Classes
A. No macroscopic pathological changes.	1. No macroscopic lesions. Gain in body weight. 2. No macroscopic lesions. Slight loss in weight.
B. Slight pathological changes.	1. Few (less than 50) small lesions (0.5 to 3 mm. in diameter) Gain in weight. 2. Few (less than 50) small lesions (0.5 to 3 mm. in diameter). Weight loss. 3. Few (less than 50) lesions, mostly small with a few larger ones. Weight loss.
C. Moderate pathological changes.	1. Numerous (more than 50) small lesions (0.5 to 3 mm. in diameter). Weight gain. 2. Numerous (more than 50) small lesions (0.5 to 3 mm. in diameter). Weight loss. 3. Numerous lesions (more than 50). Mostly small with a few larger ones. Weight loss.
D. Intense pathological changes.	1. Large lesions (less than 50), some extending around the circumference of the cecum. Marked loss in weight. 2. Many large lesions (too numerous to count) covering entire cecum. Also extensive superficial erosion. Marked loss in weight. 3. Few macroscopic lesions but intense superficial erosion of the mucosa. Marked loss in weight.

RESULTS

Pathogenicity. A total of 37 strains of *E. histolytica* were studied. Table 1 lists these strains by numbers and indicates the mean size of each. Brief clinical histories of the individuals from whom the strains were obtained also are given.

TABLE 1

Clinical histories of the patients from whom the strains of Entamoeba histolytica were obtained

Strain	Mean size in microns (Trophozoites)	Proctoscopic examination	G.I. symptoms and physical examination
		Group 1	
9	15.1	Negative	Negative
17	16.9	Ulcers in sigmoid and rectum	Amebic dysentery. Severe bloody diarrhea for three weeks.
26	16.6	Small lesions	Amebic dysentery. Diarrhea with trophozoites containing R.B.C. in stool.
28	17.7	Negative	Amebic dysentery. Diarrhea with trophozoites containing R.B.C. in feces. Amebic granuloma in colon.
39	15.2		Amebic dysentery.
		Group 2	
2		Negative	Negative
5		Negative	Negative
12	15.8	Negative	Negative
16	14.6	Negative	Negative
20	16.1	Negative	Negative
23	15.6		
24	16.3	Negative	Negative
25	16.0	Negative	Negative
29	18.0		Amebic liver abscess. No diarrhea or dysentery.
30	15.2		Negative
31	15.3	Negative	Negative
32	15.7	Negative	Negative
36	14.6	Negative	Negative
43	15.7		Amebic hepatitis. Episodes of bloody diarrhea for 5 years.
		Group 3	
7		Negative	Negative
8	12.0	Negative	Negative
10	17.3	Negative	Negative
11	14.7	Negative	Negative
13	15.7	Negative	Negative
14			
21	13.5	Minute ulcers in sigmoid and rectum	
42	17.8	Negative	Negative
22	16.1	Negative	Negative
34	10.0	Negative	Negative
33	9.5	Negative	Negative
44	8.8	Negative	Negative
37	8.6	Negative	Negative
15	8.3	Negative	Negative
35	8.3	Negative	Negative
41	8.0	Negative	Negative
38	7.7	Negative	Negative
40	7.5	Negative	Negative

These strains are divided into three groups on the basis of the severity of the infection produced in rabbits. The five strains 9, 17, 26, 28, 39 of Group 1 proved to be the most pathogenic; of 104 rabbits inoculated, 88.4 per cent developed lesions (Table 2). Four of these five strains (Table 1) produced amebic dysentery

TABLE 2

The number and percentage of rabbits infected with the three groups of Entamoeba histolytica *showing macroscopic lesions*

	Number of rabbits inoculated	Number of rabbits which developed lesions	Per cent of rabbits with lesions
Group 1 Strains 9, 17, 26, 28, 39.	104	92	88.4
Group 2 Strains 2, 5, 12, 16, 20, 23, 24, 25, 29, 30, 31, 32, 36, 43.	188	85	45.2
Group 3 Strains 7, 8, 10, 11, 13, 14, 15, 21, 22, 23, 34, 35, 37, 38, 40, 41, 42, 44.	156	0	0

in human beings. Of the rabbits inoculated with these strains, a large proportion developed large, irregular-shaped lesions, which were so numerous that they could not be counted. These lesions coalesced and crater-like ulcers often completely encircled the cecum. As shown in Table 3 the infections continued to be severe whether derived from the original cultures or from cultures taken after several passages through rabbits. The letters and numbers indicate the degree of infection (see key given above). The pathogenicity was not reduced in strain 17 even after 8 months of continuous culturing without animal passage. The degree of pathological change observed is shown in Graph 1. It will be noted that the majority of the rabbits in Group 1 developed intense pathological changes.

Group 2 is composed of strains 2, 5, 12, 16, 20, 23, 24, 25, 29, 30, 31, 32, 36, 43. These were less virulent than those of Group 1. Of the 188 rabbits inoculated, 45.2 per cent showed macroscopic lesions. The ulcers were small in practically all of the rabbits (from 0.5 to 3 mm. in diameter). The severity of the infection following the original inoculation and from rabbit to rabbit is shown for four of these strains in Table 4, while the extent of pathological changes for all of these strains is shown in Graph 1. None of the rabbits developed severe infections. A small number showed moderate pathological changes, while most of them had few or no macroscopic lesions.

Group 3 is composed of strains 7, 8, 10, 11, 13, 14, 15, 21, 22, 33, 34, 35, 37, 38, 40, 41, 42, 44; none of these produced any evidence of macroscopic lesions in 156 rabbits inoculated. No symptoms of amebic dysentery were found in any of the 18 humans from whom these strains were obtained. In one patient minute ulcers were observed in the sigmoid and rectum.

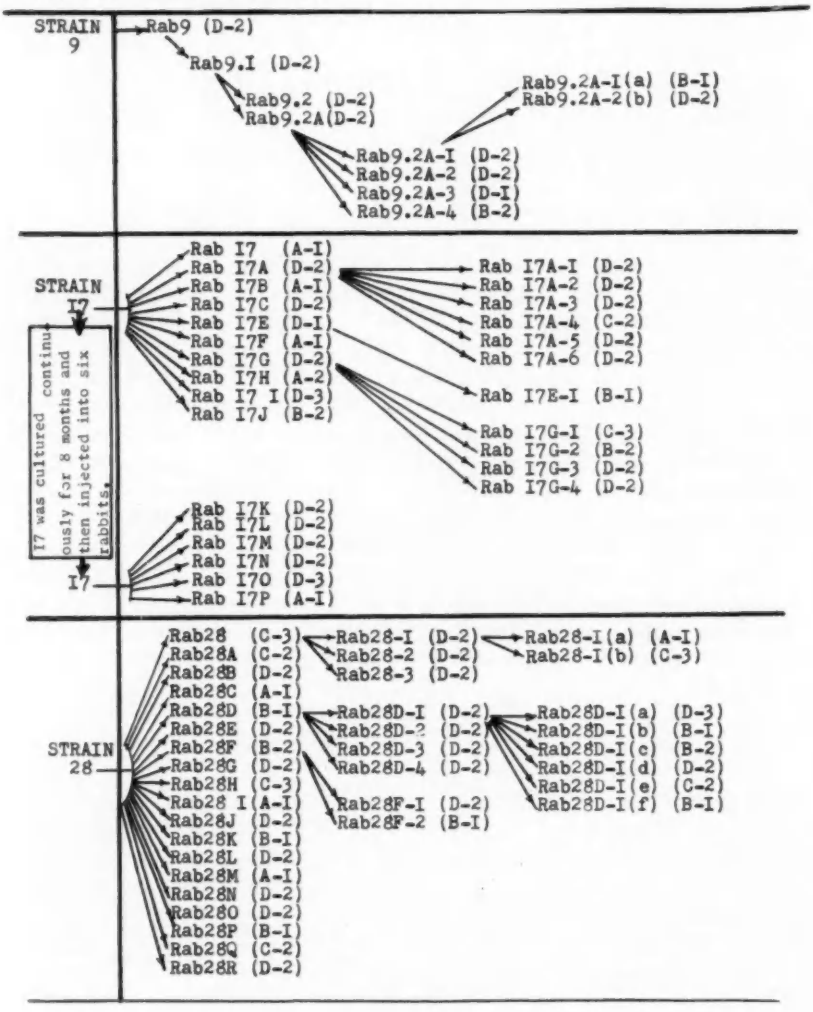
Weight Changes. The weight loss was very marked in the rabbits infected by the strains of Group 1. Table 5 gives the data for two strains of this group. In these two strains the average loss per rabbit ranged from 0.06 to 1.93 ounces per day. The average loss for all 33 rabbits with lesions was 0.98 ounces per day. Only two of the rabbits with lesions showed a gain in weight. One rabbit (9.1, lost 51 per cent of its body weight in 21 days.

Table 6 shows the weight changes in animals inoculated with three strains of

TABLE 3

Summary of pathological changes observed in rabbits infected with group 1 strains of Entamoeba histolytica

The letters with numbers in parentheses represent degrees of pathology in the rabbits; see key under Materials and Methods.

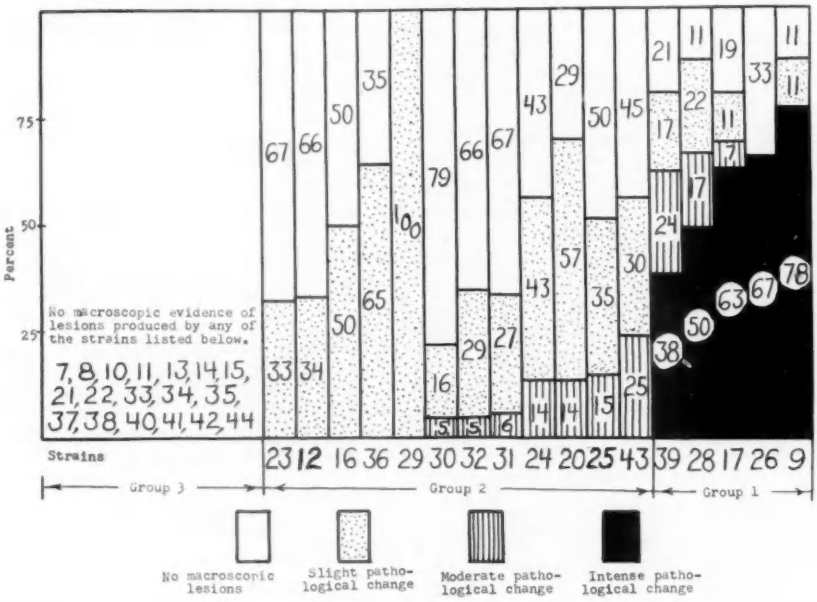


Group 2. The average weight loss for 26 rabbits that had macroscopic lesions was 0.3 ounces per day, only a third of that found in Group 1. All of the 29 rabbits with no evidence of lesions showed a weight gain.

Of the 82 rabbits of Group 3 only seven lost a very little weight while 75

GRAPH 1

Degree of pathological changes produced by strains of E. Histolytica *in rabbits*


Percent
75
50
25
No macroscopic evidence of lesions produced by any of the strains listed below.
7, 8, 10, 11, 13, 14, 15, 21, 22, 33, 34, 35, 37, 38, 40, 41, 42, 44
Strains
23 12 16 36 29 30 32 31 24 20 25 43 39 28 17 26 9
Group 3
Group 2
Group 1
No macroscopic lesions
Slight pathological change
Moderate pathological change
Intense pathological change

gained an average of 0.48 ounces per day. This gain, however, is not as much as that of the control group which gained an average of 0.8 ounces per day. No doubt the procedure of the laparotomy caused the rabbits to lose some weight. Laparotomy was not performed on the control rabbits.

Distribution of the Lesions. The cecum was found to be the most common site for lesions (Table 7). This location revealed pathological changes in approximately 98 per cent of the rabbits infected with strains of either Group 1 or Group 2. However, there is a difference between these two groups in regard to the spread of the lesions from the cecum to other parts of the digestive tract. In the infections caused by strains of Group 1, for example, 46.7 per cent had lesions in the appendix, 53.3 per cent had lesions in the colon, and 10 per cent had lesions in the ileum. With infections caused by the strains of Group 2, on the other hand, the corresponding values are 15.4 per cent for the appendix, 11.0 per cent for the colon, with no evidence of pathological changes in the ileum.

Comparison of Size of Trophozoites in Culture and in Lesions. Average size was determined for trophozoites of 12 strains in cultures just prior to inoculating them into rabbits, from the lesions in the rabbit, and subsequently from cultures obtained from the digestive tract of the infected rabbits. It can be seen (Table 8) that in all 12 strains studied there was an increase in the size of the trophozoites in the ulcers when compared with their size in culture. Furthermore when cul-

TABLE 4

Summary of pathological changes in rabbits infected with Entamoeba histolytica *strains 24, 25, 36 and 32 (group 2) which produced mild ulceration*

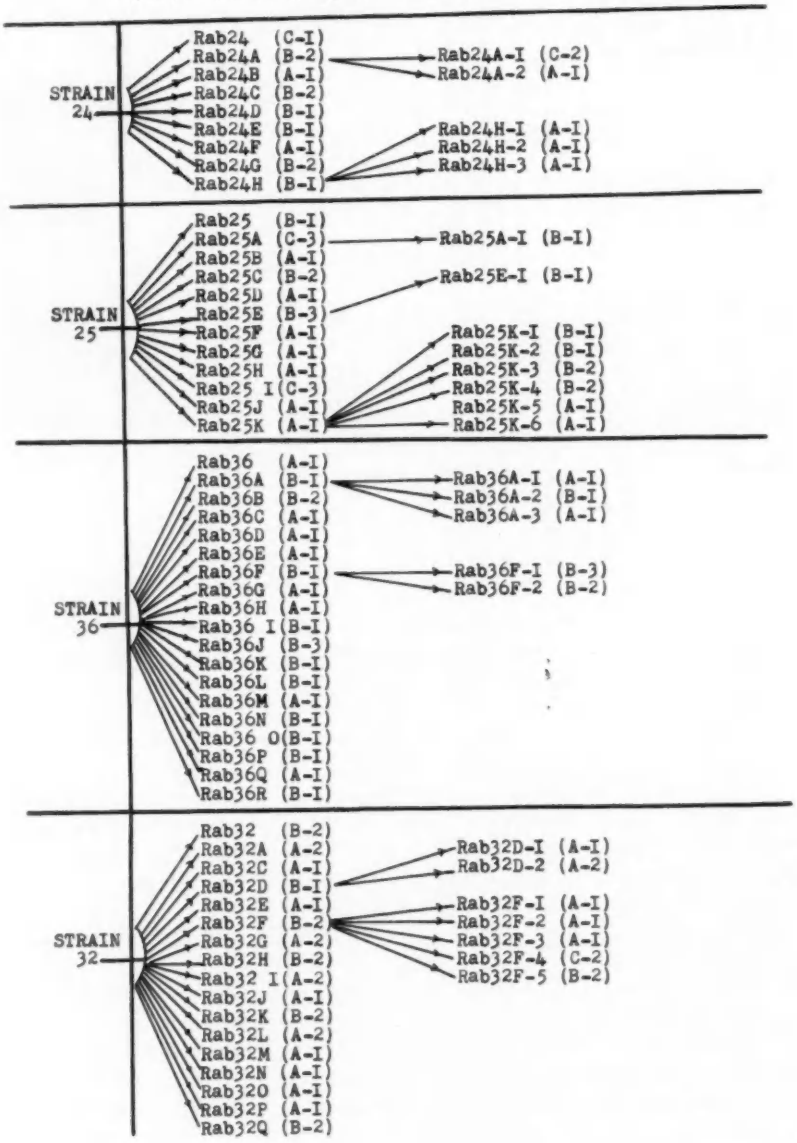


TABLE 5

Weight changes observed in rabbits inoculated with strains 9 and 17 (Group 1)

Rabbit number	Weight loss			Weight gain
	Mean loss per day, ounces	Body weight lost		Mean gain per day, ounces
		Per cent	Days	
9	1.6	40	14	
9.1	1.17	51	21	
9.2	0.7	25	18	
9.2A	0.06	2	18	
9.2A-1	1.18	46	22	
9.2A-2	0.5	5	4	
9.2A-3	1.14	16	7	
9.2A-4	0.43	6	7	
9.2A-I(a)	—	—	—	0.57
9.2A-I(b)	1.25	24	8	
17*	—	—	—	0.25
17A	1.36	30	11	
17B*	—	—	—	0.2
17C	0.8	25	16	
17E	1.1	14	5	
17F*	—	—	—	0.6
17G	1.63	36	19	
17H	0.75	14	12	
17I	0.65	16	10	
17J	0.73	17	11	
17K	1.6	46	17	
17L	1.08	39	17	
17M	1.35	45	17	
17N	1.3	48	19	
17O	1.2	35	17	
17P*	—	—	—	0.57
17A-1	1.1	14	5	
17A-2	1.75	29	6	
17A-3	1.6	18	5	
17A-4	0.55	16	11	
17A-5	1.63	43	22	
17A-6	1.16	27	12	
17E-1	—	—	—	0.12
17G-1	0.92	18	19	
17G-2	0.26	5	19	
17G-3	1.8	39	19	
17G-4	1.93	27	14	

* No lesions.

Average loss per day for all 33 rabbits = 0.98 oz.

TABLE 6

Weight changes in rabbits inoculated with strains 24, 25, 32, and 36 (Group 2)

Rabbit number	Weight loss			Weight gain
	Mean loss per day, ounces	Body weight lost		Mean gain per day ounces
		Per cent	Days	
24	0.86	14	15	
24A	0.72	6	9	
24B*	—	—	—	0.56
24C	0.61	13	13	
24D	—	—	—	0.26
24E	—	—	—	0.12
24F*	—	—	—	0.3
24G	0.79	22	14	
24H	0.2	9	15	
24A-1	—	—	—	0.06
24A-2*	—	—	—	0.28
24H-1*	—	—	—	0.62
24H-2*	—	—	—	0.3
24H-3*	—	—	—	0.24
25A	0.57	22	22	
25B*	—	—	—	0.59
25C	0.17	7	20	
25D*	—	—	—	0.15
25E	0.7	21	20	
25F*	—	—	—	0.35
25G*	—	—	—	1.0
25H*	—	—	—	1.13
25I	0.13	5	15	
25J*	—	—	—	0.23
25K*	—	—	—	0.2
25E-1	—	—	—	0.4
25K-1	—	—	—	0.7
25K-2	—	—	—	0.1
25K-3	0.1	1	16	
25K-4	0.18	6	16	
25K-5*	—	—	—	0.4
25K-6*	—	—	—	0.53
32	0.12	3	12	
32C*	—	—	—	0.56
32D	—	—	—	0.5
32E*	—	—	—	0.37
32F	0.31	10	16	
32G*	0.37	10	16	
32H	0.44	10	16	
32J*	—	—	—	0.18
32K	0.06	2	16	
32L*	0.1	2	16	
32M*	—	—	—	0.38
32N*	—	—	—	0.18
32O*	—	—	—	0.18
32P*	—	—	—	0.65
32Q	0.35	10	17	
32D-1*	—	—	—	0.23
32D-2*	—	—	—	0.3
32F-1*	—	—	—	0.62
32F-2*	—	—	—	0.83
32F-3*	—	—	—	0.25
32F-4	1.5	30	12	
32F-5	0.86	23	12	

* No lesions

Average loss per day for 26 rabbits. 0.3 ounces

TABLE 7

Distribution of the lesions in the digestive tract of the rabbit (Percentage of infected rabbits with lesions in each location)

	Cecum	Appendix	Colon	Ileum	Number of rabbits
Group 1 Strains 9, 17, 26, 28, and 39.	97.8%	46.7%	53.3%	10.0%	128
Group 2 Strains 2, 5, 12, 16, 20, 23, 24, 25, 29, 30, 31, 32, 36, and 43.	98.4%	15.4%	11%	0	76

TABLE 8

Sizes of trophozoites in cultures; in lesions from inoculated rabbits; and in cultures isolated from digestive tracts of the infected rabbits

Strain	Sizes of trophozoites in cultures before rabbit injections		Sizes of trophozoites from lesions of rabbits' digestive tract		Sizes of trophozoites in cultures incubated from the infected rabbits	
	Mean size in microns	Number measured	Mean size in microns	Number measured	Mean size in microns	Number measured
9	15.13	122	19.85	94	15.29	89
17	16.90	559	19.12	589	16.82	482
26	16.60	57	21.55	49	16.65	52
28	17.67	208	21.95	167	17.76	65
16	14.60	84	19.75	105	16.85	135
24	16.30	33	17.59	45		
25	16.00	204	17.59	102	16.07	112
28	17.67	208	21.95	167	17.76	65
30	15.16	123	17.01	73	15.16	85
36	14.60	250	18.77	183	15.55	185
39	15.20	111	18.18	401		
43	15.70	65	16.80	24		

tures were subsequently made from the lesions, the trophozoites returned to the original size found in culture before injection into rabbit.

OBSERVATIONS AND PATHOLOGIC FINDINGS

Group 1. Within a few days after inoculation, the rabbits infected with the strains of Group 1 lost their appetites, became hypoactive, and developed severe diarrhea with demonstrable trophozoites in the feces. A small number of the rabbits showed macroscopic evidence of blood in feces. Every rabbit with lesions suffered a marked loss of body weight. On autopsy the intestinal contents were found to be very soft or watery, containing large quantities of frothy mucus. Many of the rabbits had gas in the digestive tract. Hyperemia of the cecum and the colon was common. In many cases the cecum was shrunken and the wall greatly thickened, especially the submucous layer (Figs. 1 and 2). A large number of lesions was found in most of the rabbits. The lesions in the cecum and the colon were irregular in shape, crater-like, and large, often extending completely

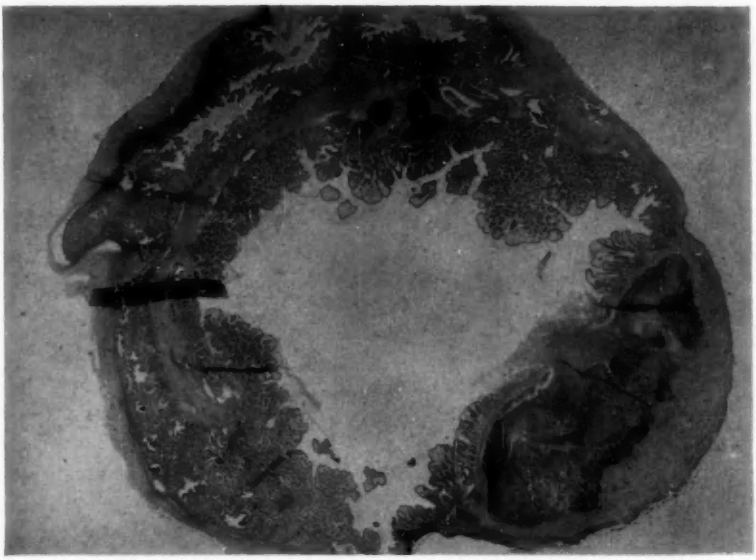


FIG. 1. Section of cecum (rabbit 17 C) showing a large crater-like ulcer and a few smaller lesions, 16 days after infection. Note the marked thickening of the cecal wall.

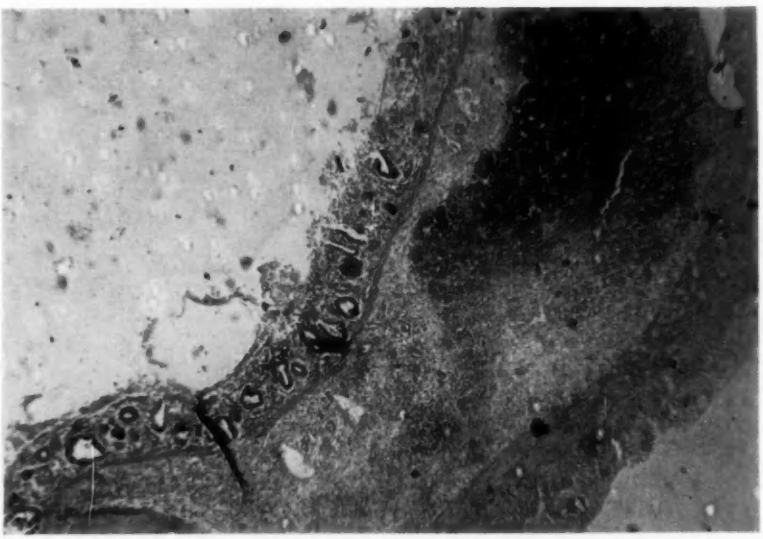


FIG. 2. Section of cecum (rabbit 17 J) showing large ulcer in the thickened submucosa and also extensive superficial erosion of the mucosa.

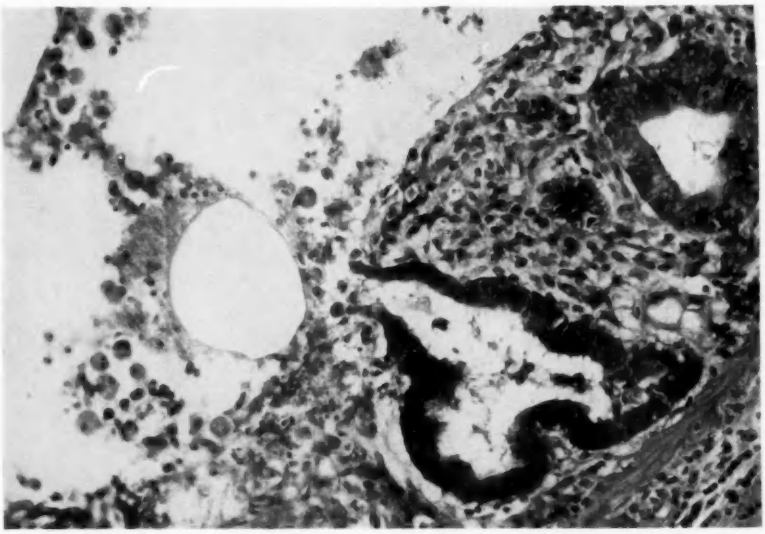


FIG. 3. High power magnification of the mucosal surface of the cecum of Fig. 2. Notice many amoebae on the eroded surface of the mucosa.

around the intestine. They frequently contained a cream colored exudate. The ulcers extended to the base of the submucosa but not into the muscle layer. No perforations were found, even in the most severe fulminating cases. In addition to the deep ulcers, there was extensive erosion of the mucosa (Figs. 2 and 3). Scrapings from the mucosa or from the deep lesions contained huge numbers of active trophozoites with ingested red blood cells.

Sections were made of the digestive tracts of 21 rabbits infected with strains 9, 17, 28, and 39 of Group 1. Infiltration by lymphocytes and plasma cells was observed in a few infections. In 19 of the 21 rabbits, eosinophilic response was observed. The eosinophils were found in the mucosa and submucosa, usually in the uninvaded tissues adjacent to the lesions. In 10 of the 19 rabbits the eosinophilic response was intense, in four it was moderate, and in five it was light. A section of the cecum of one rabbit (28 P) showed an ulcer containing a purulent exudate composed almost entirely of eosinophils (Figs. 6 and 7); the submucosa contained a huge number of these cells. It is held by other investigators that the eosinophilic response in the rabbit corresponds to neutrophils in man, i.e., in rabbits neutrophils look like eosinophils in man. Sections of the cecum were made of two uninfected controls; no eosinophils were found in one rabbit while very few (the normal number) were seen in the second one.

The lesions in the appendix and in the small area of the cecum adjacent to the sacculus rotundus were consistently small (0.5 to 2 mm. in dia.), and macroscopically they appeared round but upon microscopic examination in section they proved to be flask-shaped, extending to the base of the submucosa (Figs. 4

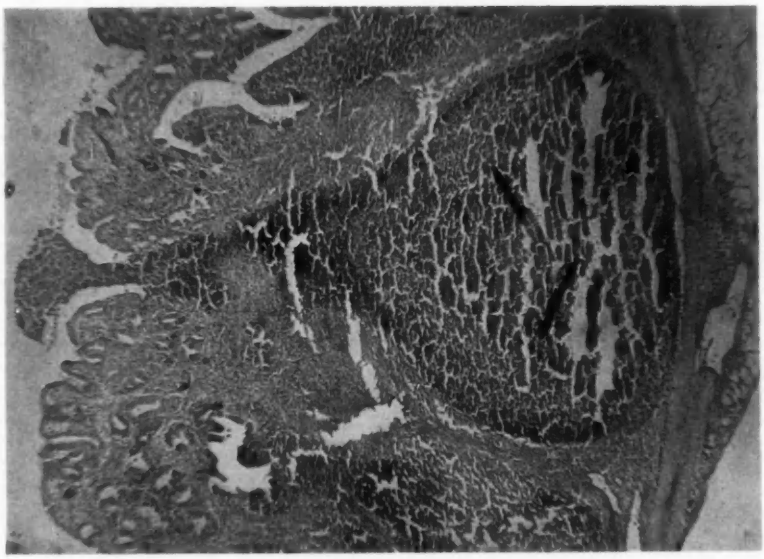


FIG. 4. Section of appendix (rabbit 39 A) showing a flask-like ulcer. 15 days after infection.

FIG. 5. Section of cecum adjacent to sacculus (rabbit 39 I) showing bottle-shaped ulcers which extend to the base of the submucosa. Notice marked destruction of the mucosa. Many amoebae found in the lesions.

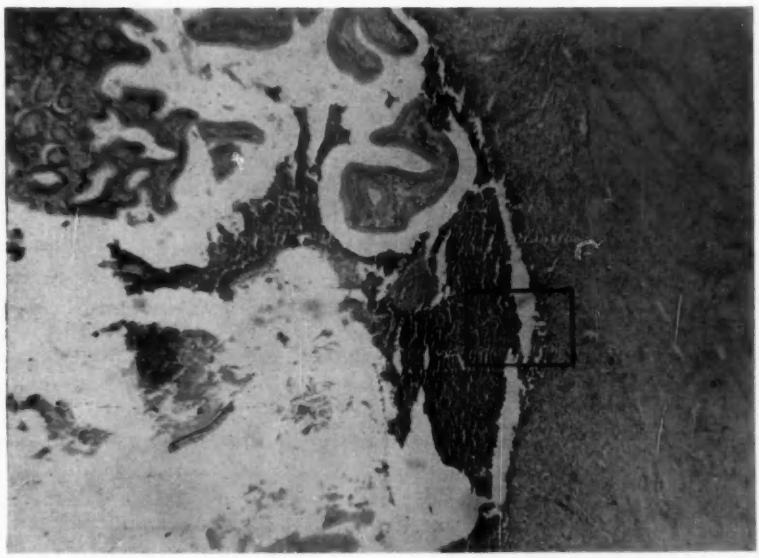


FIG. 6. Section of cecum (rabbit 28 P) showing a 12-day old lesion with a considerable amount of purulent exudate.

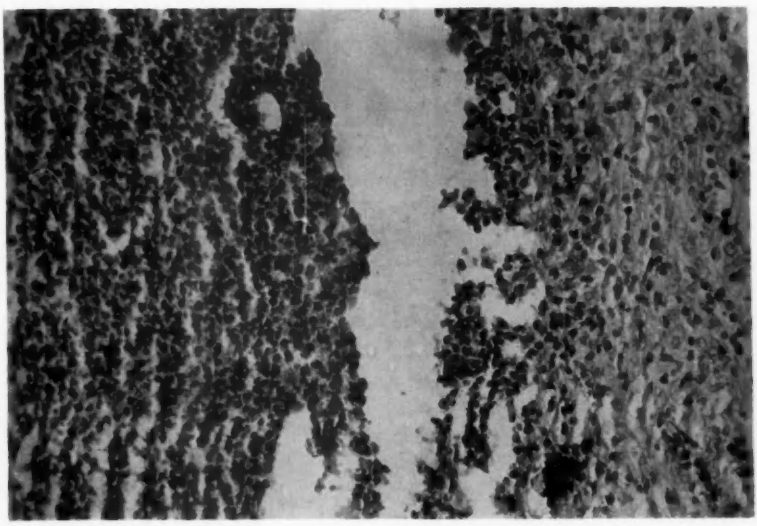


FIG. 7. High power magnification of the marked area of Fig. 6. The purulent exudate shown on the left half of the figure is almost entirely made of eosinophils. On the right side of the figure notice intensive invasion of eosinophils in the submucosa.

and 5). The ulcers in the ileum also were small; they usually were located in the lower ileum, although in two rabbits they were found in the upper half of the small intestine. There was no macroscopic evidence of amebic lesions in the liver of any of the rabbits in this study.

Group 2. As mentioned above, the strains in this group were less pathogenic than those of Group 1. Only a few of the rabbits suffered from diarrhea, which was of a mild nature. Almost consistently the lesions were round and small (0.5 to 3 mm. in diameter). The weight loss was less than one-third of that of Group 1. In most of the rabbits the intestinal contents were normal or slightly soft, and only an occasional one had watery contents. The most common site for lesions was a small area of the cecum adjacent to the sacculus rotundus. Fewer trophozoites as well as a smaller number with red blood cells were found when compared with the highly virulent strains of Group 1. Micropathological studies were made on 15 rabbits infected with five strains. In 13 of these rabbits eosinophilic response was evident and to the same degree as in the rabbits infected by Group 1 strains. In 9 of the 13, the response was intense, in three it was moderate, and in one it was light.

Group 3. None of the 156 rabbits infected by the 18 strains of this Group developed any evidence of macroscopic lesions; no diarrhea developed and the intestinal contents were normal in all of the rabbits. A gain in weight occurred in 91 of the rabbits while a few showed a very slight loss of weight. That the amebae lived and multiplied in these rabbits was demonstrated by recovering the amebae in cultures made from intestinal scrapings at autopsy of most of the rabbits; in three of the rabbits 32 days after infection, parasites were found in the cultures, yet no macroscopic lesions were present in these rabbits.

DISCUSSION

The findings in this report indicate that there are definite differences in the pathogenicity of various physiological strains of *E. histolytica* and that their virulence remains fairly constant, as shown by the fact that any one strain produces a similar degree of pathological change fairly consistently when injected into a large number of rabbits. In one series of experiments the amebae were cultured for eight months without animal passage, and then were found to produce the same degree of pathology as they did originally. Variation in the pathogenicity of strains of *E. histolytica* has been shown to occur by other investigators, among them Faust (1946), and Meleney and Frye (1933, 1935, 1936, 1937a). Meleney and Frye infected kittens and found that a lower rate of infection and less intense pathological changes were produced by two strains from healthy carriers from the hill country of middle Tennessee than by two strains from active cases of amebic dysentery from the bottom land of west Tennessee. Even after five years of cultivation and intermittent transfers to kittens each strain still maintained its original pathogenic index.

The distribution of lesions in rabbits infected with *E. histolytica* closely follows the pattern observed in man. In human infections, advanced pathological changes are most frequently found in the cecum and ascending colon. The same

distribution was observed in rabbits used in the present study. The cecum was the most frequent location for lesions in these animals, while the ascending colon was ulcerated in more than one-half of the rabbits that showed lesions. One-third of those with lesions in the ascending loop also had ulcers throughout the entire length of the colon. Ulcers in the ileum were generally located at the terminal end; however, two rabbits with severe infections had lesions in the upper half of the small intestine.

Eosinophilic response was marked in the rabbits showing ulceration. Sections of the intestinal tract of 36 rabbits studied, showed eosinophilic response in all except four; of these reactions 19 were intense, 7 moderate, and 6 light.

A considerable amount of work has been published on the sizes of *E. histolytica*. In the present study it can be seen (Table 1) that there is no significant difference in the sizes of the strains of Group 1, Group 2 and the first seven strains of Group 3. All of the highly virulent strains of Group 1 were found to be larger than 10 microns in size and the four human cases from whom they were isolated suffered from amebic dysentery. On the other hand, none of the nine strains less than 10 microns in size produced any macroscopic lesions in the rabbit and none of the human beings from whom these strains were isolated showed any symptoms of amebic dysentery.

We also observed a distinct difference in the culturability and behavior of the strains which were 10 microns or less in size compared with the larger strains. The larger strains were cultured satisfactorily but it was very difficult to culture the small strains. Often the cultures of the latter would remain negative for many days and then a few of the tubes would suddenly show large numbers of trophozoites. These trophozoites were very sluggish when compared with those of the larger strains. These observations have also been made by Spector (1936) who found that the small strains grew infrequently and with difficulty in Cleveland-Collier medium. She states that in human beings, the symptoms produced by the small strain were much milder than with the larger strains and also that liver and lung abscesses were not produced by the small strains. Hoare (1950) believes that the small race of *E. histolytica* is quite harmless to man. Frye and Meleney (1938), after infecting kittens with a small strain of *E. histolytica*, concluded that it was distinctly less virulent than the larger strains. Drbohlav (1925) cultured a small strain of *E. histolytica* but noted that this strain did not ingest red blood cells. Moreover, although it could be established in kittens. it never caused dysentery or ulceration of the large intestine.

The question of whether *E. histolytica* is an obligatory parasite or is capable also of living as a commensal in the intestinal lumen has been discussed. Some workers, Meleney and Frye (1937a), Meleney (1944), and Hoare (1950 and 1952) believe that *E. histolytica*, although capable of invading tissues of the host and producing lesions, in most cases does not manifest evidence of virulence and is capable of living in the gut as a harmless commensal. Other investigators do not accept the commensal role of *E. histolytica* and believe this parasite is an obligatory one. In the present study it appears that the 18 strains of Group 3 (some larger than 10 and others 10 microns or less in size) lived and multiplied in the

rabbits as commensals in which they produced no symptoms or pathological changes.

There is a wide difference of opinion as to whether all individuals who show the presence of *E. histolytica* should be treated. It is known that not more than 10 per cent (most likely less) of human beings with *E. histolytica* ever suffer appreciable symptoms from the infection. Some believe that all cases should be treated regardless of whether they show clinical symptoms or not and whether they harbor large or small strains. Hoare (1950) believes that in actual practice only those cases with clinical symptoms should undergo treatment, and that infections with the small strains may be ignored. It appears from our studies that the strains 10 microns or less in size play a minor role, if any, in producing clinical amebiasis.

SUMMARY

The pathogenicity of 37 strains of *E. histolytica*, isolated from human patients and carriers, was studied in white rabbits. Five of these strains (Group 1) consistently produced severe ulceration in 88.4 per cent of the rabbits inoculated, with crater-like lesions, intense diarrhea, marked loss of weight and huge numbers of trophozoites containing red blood cells. Four of the 5 patients from whom these strains were isolated suffered from amebic dysentery. Group 2, comprising 14 strains, was mildly pathogenic producing only small lesions in 45.2 per cent of the rabbits inoculated, which lost little weight. Two of the patients furnishing these strains had amebic liver abscesses with few or no intestinal symptoms; the rest were asymptomatic. The 18 strains of Group 3, secured from asymptomatic carriers, were also nonpathogenic to rabbits.

The trophozoites of all the highly virulent strains of Group 1 were larger than 10 microns, but there were also large amebae in Group 3; none of the strains measuring less than 10 microns (size of trophozoites in culture) produced macroscopic evidence of lesions in rabbits. Amebae multiplying in ulcers were invariably larger than those in the cultures from which they were derived, but reverted to their former size when cultures were made from the lesions.

The lesions in infected rabbits showed only occasional infiltration by lymphocytes and plasma cells, but in all but 4 of the 36 animals examined there was a characteristic invasion of eosinophils which are believed to correspond to the neutrophils of man.

REFERENCES

Drbohlav, J. J., 1925. Culture d'Entamoeba dysenteriae type *Tetragena minuta*, *Ann. Parasitol.* **3:** 358–360.

Faust, E. C., Heilbrunn, L., Lewis, R., and Murray, M. L., 1946. Difference in culturability, infection, and pathogenicity of human strains of *Endamoeba histolytica*, *Proc. Soc. Exper. Biol. and Med.* **63:** 270.

Frye, W. W., and Meleney, H. E., 1938. The pathogenicity of a strain of small race of *Endamoeba histolytica*, *Amer. J. Hyg.* **27:** 580–589.

Hoare, C. A., 1950. Amoebiasis in Great Britain, with special reference to carriers, *British Med. Jour.* **2:** 238–247.

HOARE, C. A., 1952. Parasitological Reviews. The commensal phase of *Entamoeba histolytica*, *Exp. Parasitology*. **1:** 411–427.

LAIRD, R. L., DRINNON, V. P., AND DAVIS, A. B., 1949. Routine culture methods in diagnosing *Endamoeba histolytica*, *Nat. Mal. Soc.* **8:** 198–201.

MELENEY, H. E., 1944. The relationship of clinical amebiasis to various strains and growth requirements of *Endamoeba histolytica*, *Puerto Rico J. of Public Health and Trop. Med.* **20:** 59–70.

MELENEY, H. E., AND FRYE, W. W., 1933. Studies of *Endamoeba histolytica* and other intestinal protozoa in Tennessee; V. a comparison of five strains of *Endamoeba histolytica* with reference to their pathogenicity for kittens, *Am. J. Hyg.* **17:** 637–655.

MELENEY, H. E., AND FRYE, W. W., 1935. Studies of *Endamoeba histolytica* and other intestinal protozoa in Tennessee; IX. further observations on pathogenicity of certain strains of *E. histolytica* for kittens, *Am. J. Hyg.* **21:** 422–437.

MELENEY, H. E., AND FRYE, W. W., 1936. Pathogenicity of *Endamoeba histolytica*, *Tr. Roy. Soc. Trop. Med. and Hyg.* **29:** 369.

MELENEY, H. E., AND FRYE, W. W., 1937a. Effect of direct animal passage on pathogenicity of *Endamoeba histolytica* for kittens, *Amer. J. Hyg.* **25:** 313.

SPECTOR, B. K., 1936. Significance of the small variety of *Endamoeba histolytica*, *Amer. J. Pub. Health* **26:** 813–818.

TOBIE, J. E., 1949. Experimental infection of the rabbit with *Endamoeba histolytica*, *Amer. J. Trop. Med.* **29:** 859–870.

WESTPHAL, A., 1941. Experimentelle Amöbenruhr beim Kaninchen, *Deut. Trop. Med. Ztschr.* **45:** 653–657.

THE PHARMACOLOGICAL BASIS FOR THE PROLONGED ANTIMALARIAL ACTIVITY OF PYRIMETHAMINE[1]

CARL C. SMITH AND JEAN IHRIG

The Christ Hospital Institute of Medical Research, Cincinnati, Ohio

Carefully controlled studies on human volunteers (Coatney *et al.*, 1953) have shown that a single 25 mg. dose of pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) protects against subsequent infection with trophozoites of *Plasmodium vivax* for a period of at least 17 days. In numerous field studies, suppression of both *P. vivax* and *P. falciparum* infections has been achieved by administration of 25 mg. doses of pyrimethamine once weekly. In addition, Schmidt and Fradkin (1956, to be published) in this laboratory have obtained similar though less protracted protection against infection with *P. cynomolgi* in rhesus monkeys following single oral doses of 1 mg. per kg.

Little serious attention has been given to the basis for this protracted protective action. Preliminary studies by Goodwin (1952) suggested that the prolonged effectiveness of pyrimethamine might be due to formation of a metabolic product which persisted in the body long after the parent drug had been excreted. The evidence for this suggestion came from work on a small group of human volunteers who ingested single 100 mg. doses. The concentration of chemically determinable pyrimethamine in the sera of these subjects reached a peak within two hours, declined precipitously thereafter, and fell below detectable levels eight to twelve hours after drug administration. However, as the concentration of chemically determinable pyrimethamine declined, there appeared to be an increase in serum content of materials which blocked the utilization of folic acid by *Streptococcus fecalis*. These folic acid antagonists persisted in the serum at relatively high concentrations for twenty-four to forty-eight hours.

The present study, a systematic investigation of the metabolic fate of pyrimethamine in man and rhesus monkey, was designed to test the hypothesis that the prolonged antimalarial activity of pyrimethamine might reside in metabolic conversion of this pyrimidine to folic acid antagonists which were more active and more slowly excreted than the parent compound.

MATERIALS AND METHODS

General. Urinary excretion of pyrimethamine[2] after oral administration was investigated in eight healthy human volunteers; in six the dosage was 25 mg. twice daily for two days, in two 50 mg. twice daily for two days. Twenty-four hour urine collections were made for two days preceding treatment, for five days after treatment and on days 8, 11, 15 and 16. Urine samples were acidified to pH 4 with HCl as collected and stored at 6°C. An aliquot of each twenty-four

[1] This study was supported in part by a research grant E824 from the National Microbiological Institute, National Institutes of Health, U. S. Public Health Service.

[2] We are indebted to Dr. George Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y., for the pyrimethamine used in these studies.

hour collection was sterilized by filtration through sintered glass; the remainder was stored at −20°C.

Serum levels of pyrimethamine following oral administration were studied in two human subjects. Blood samples were obtained at 4, 8, 24, 72, 120 and 168 hours after a single 100 mg. dose of drug.

The urinary excretion of pyrimethamine was also studied in a group of six, 3- to 4-kilogram rhesus monkeys. In a preliminary study in which the animals were fed the mixed diet normally used in this laboratory (Schmidt and Genther, 1953a), large amounts of folic acid-like materials, which interfered with the microbiological estimation of folic acid antagonists were excreted in the urine. Since these interfering substances could be reduced to a low level by feeding boiled rice, egg powder, peanuts and ascorbic acid, this restricted diet was employed in all subsequent experiments. Pyrimethamine was administered orally in doses of 5 mg. per kg. once daily on two successive days. Twenty-four hour urine specimens, free of fecal material, were collected in ice baths for two days preceding treatment, during treatment and for four days after the last dose of drug. These specimens were processed in the same way as the human urine samples.

The same group of six monkeys was used for studying serum levels following pyrimethamine administration. The drug was administered orally in doses of 5 mg. per kg. body weight once daily on two successive days. Blood samples were obtained two hours after each dose and at 1, 2, 3, 5 and 7 days after the last dose of drug.

Analytical procedures. Chemical. Urine aliquots were analyzed for pyrimethamine content by the following modification of the method of Schmidt *et al.* (1953b). Five ml. of urine were made alkaline by the addition of 1 ml. of 1 N NaOH and shaken for ten minutes with 25 ml. of ethylene dichloride (EDC) containing 2 per cent ethyl alcohol. After centrifugation the aqueous phase was removed by aspiration and a 10 ml. aliquot of the EDC phase was shaken for ten minutes with 5 ml. of 0.2 N HCl. Following centrifugation of the mixture, the optical density of the acid phase was measured at 270 mμ in a Beckman Model DU spectrophotometer. With this method known amounts of pyrimethamine added to control urine could be recovered with an error of less than four per cent.

Control (and treatment) urine contained certain non-specific EDC-soluble material, which also absorbed at 270 mμ. In the human subjects the daily excretion of this material was equivalent to approximately 5 mg. of pyrimethamine, the amount varying somewhat in different subjects. In monkeys receiving the special diet the daily excretion approximated 0.3 mg. of pyrimethamine. A correction factor representing the mean daily excretion of this blank material during two (monkey) or four (human) twenty-four hour control periods was determined for each subject. This factor was utilized to derive the true pyrimethamine content of urine samples collected during treatment periods.

Serum concentrations of pyrimethamine were determined by the colorimetric method described by Schmidt *et al.* (1953b).

Microbiological. The pyrimethamine content of serum and urine was estimated microbiologically using *Streptococcus fecalis* (A.T.C.C. No. 8043) as the test organism (Hitchings, 1952). Sterile serum and urine samples were diluted in two-fold steps from 1:5 to 1:1280, one ml. of each dilution being added to 9 ml. of medium (A.O.A.C. medium for folic acid assay to which glucose and folic acid (0.02 γ) were added separately). The tubes were inoculated with 0.1 ml. of a standardized suspension of twice washed organisms and incubated for 19 to 21 hours at 37°C. Growth was estimated turbidimetrically; the concentration of growth-inhibiting ("anti-folic acid") substances expressed as pyrimethamine equivalent, was estimated by comparing the curve derived from a series of urine dilutions with the similarly shaped curve obtained when decreasing amounts of pyrimethamine (0.16 to 0.005 γ per tube) were added to the assay medium. The growth-inhibiting activity of the parent drug was determined in each assay. Duplicate assays were carried out on each serum and urine sample; in most instances the individual estimates did not differ from their mean by more than ± 10 per cent.

RESULTS

Data on the urinary excretion of pyrimethamine (and closely related metabolic derivatives) in human subjects have been summarized in Table 1. These data indicate that the excretion of chemically and microbiologically determinable pyrimethamine was fairly parallel. In the five days following treatment 9.5 to 14.1 per cent of the administered drug was recovered in the urine

TABLE 1

Urinary excretion of pyrimethamine after oral administration to human subjects

Subject	Method of analysis	Per cent of dose excreted on day							
		1	2	3	4	5	8	11	15
CE[1]	Chem.	1.4	3.7	1.9	1.7	2.0	2.5	0.6	—
	Micro.	2.0	3.6	2.9	2.3	2.1	1.8	0.9	—
RF[1]	Chem.	0.8	3.6	3.0	2.8	1.6	1.7	0.5	—
	Micro.	1.3	2.4	3.2	3.0	1.4	1.6	0.7	0.4
RH[1]	Chem.	1.8	4.6	2.1	1.9	1.7	1.1	<0.2	—
	Micro.	2.4	4.0	1.7	2.1	1.4	1.0	0.4	0.2
JI[1]	Chem.	4.2	4.5	1.6	2.1	1.5	1.2	<0.2	—
	Micro.	3.6	4.1	1.6	1.9	1.2	1.5	0.5	0.5
AJ[1]	Chem.	1.6	0.4	1.7	1.9	4.7	0.6	<0.2	—
	Micro.	1.1	1.0	1.3	1.3	2.5	0.8	0.4	0.2
JR[1]	Chem.	5.0	3.6	1.4	4.2	2.4	1.9	1.0	—
	Micro.	5.3	3.9	2.3	5.4	2.8	2.2	0.8	0.5
CS[2]	Chem.	2.5	3.5	3.0	3.1	2.3	1.3	—	—
	Micro.	2.4	3.1	3.8	3.2	2.5	1.7	—	—
LS[2]	Chem.	1.4	3.0	3.5	2.0	—	—	—	—
	Micro.	1.7	2.2	1.5	1.5	—	—	—	—

[1] 25 mg. twice daily on days 0 and 1.
[2] 50 mg. twice daily on days 0 and 1.

TABLE 2

Cumulative urinary excretion of pyrimethamine in human subjects

Subject	Method of analysis	Cumulative per cent of dose excreted on day				
		1	2	3	4	5
CE[1]	Chem.	1.4	4.4	6.3	8.0	10.0
	Micro.	2.0	4.6	7.5	9.8	11.9
RF[1]	Chem.	0.8	4.0	7.0	9.8	11.4
	Micro.	1.3	3.1	6.3	9.3	10.7
RH[1]	Chem.	1.8	5.5	7.6	9.5	11.2
	Micro.	2.4	5.2	6.9	9.0	10.4
JI[1]	Chem.	4.2	6.6	8.2	10.3	11.8
	Micro.	3.6	5.9	7.5	9.4	10.6
AJ[1]	Chem.	1.6	1.2	2.9	4.8	9.5
	Micro.	1.1	1.6	2.9	4.2	6.7
JR[1]	Chem.	5.0	6.1	7.5	11.7	14.1
	Micro.	5.3	6.5	8.8	14.2	17.0
CS[2]	Chem.	2.5	4.8	7.8	10.9	13.2
	Micro.	2.4	4.3	8.1	11.3	13.8
LS[2]	Chem.	1.3	3.4	5.8	7.8	—
	Micro.	1.7	3.4	5.1	6.9	—

[1] 25 mg. twice daily on days 0 and 1.
[2] 50 mg. twice daily on days 0 and 1.

by chemical assay and 6.7 to 17.0 per cent by microbiological assay (Table 2). With either method the recovery in the first five days after drug administration averaged 12 per cent. The above data show clearly that the excretion of pyrimethamine and anti-folic material was quite prolonged and relatively constant, the rate of excretion diminishing only slightly by the eighth day. Some of the subjects were still excreting significant amounts of pyrimethamine (measured chemically) eleven days after treatment and activity could still be detected after fifteen days by the more sensitive but less accurate microbiological assay. Traces (0.02 ± 0.01 per cent of the dose) of anti-folic material were found 35 to 37 days after treatment in the urine of subject C.S., who received a total dose of 200 mg.

The concentrations of pyrimethamine in serum after oral administration to two human volunteers are shown in Table 3. These data indicate a rather close parallelism between the concentrations of pyrimethamine measured by chemical and microbiological assays and offer no indication that pyrimethamine leaves the blood stream rapidly or undergoes extensive conversion to a more active folic acid antagonist as suggested by Goodwin (1952). Pyrimethamine determined by either method of analysis appears to persist in the blood for at least seven days.

Table 4 summarizes the excretory patterns of chemically and microbiologically determinable pyrimethamine in four representative monkeys fed the restricted diet. There are several noteworthy items in this table. In the first place pyrimethamine was excreted relatively rapidly during the first three

TABLE 3

Concentration of pyrimethamine in the serum of human subjects receiving a single dose of 100 mg.

Time in hours	Mg. pyrimethamine per liter serum			
	CS		LS	
	Chem.	Micro.	Chem.	Micro.
4	1.10	1.04	—	0.94
8	0.95	0.93	—	0.75
24	0.60	0.74	0.50	0.61
72	0.53	0.41	0.42	0.42
120	0.42	0.29	—	—
168	0.32	0.21	0.35	0.19

TABLE 4

*Cumulative urinary excretion of pyrimethamine in the rhesus monkey**

Monkey number	Method of analysis	Cumulative per cent of dose excreted on day				
		1	2	3	4	5
6825	Chem.	9.6	15.7	18.4	20.2	20.8
	Micro.	6.6	12.0	12.8	13.6	13.8
6827	Chem.	10.8	15.0	18.4	19.8	20.2
	Micro.	6.1	8.2	10.3	10.7	10.9
6830	Chem.	8.8	15.8	19.1	22.8	24.3
	Micro.	4.8	10.6	11.0	13.2	13.9
6831	Chem.	23.7	30.3	32.5	35.0	37.2
	Micro.	20.2	25.6	26.8	28.4	30.3

* 5 mg./kg. administered on days 0 and 1.

days, somewhat more slowly but steadily for the next several days. Secondly, within a six day period, urinary recoveries of pyrimethamine ranged from twenty to forty per cent leaving unknown the fate of some sixty to eighty per cent of the drug. In each subject, the elimination of pyrimethamine as measured microbiologically was significantly less than that measured by the extraction-ultraviolet absorption procedure. This last observation suggests that the monkey converts pyrimethamine to substances which are closely related but are less potent folic acid antagonists than the parent compound. Although it was necessary to place monkeys on a restricted diet in order to estimate pyrimethamine in urine microbiologically, the diet appeared to have no significant effect on the metabolism of pyrimethamine. The recovery of chemically determined pyrimethamine in the urine was essentially identical on either dietary regimen.

Table 5, which summarizes the serum concentrations of pyrimethamine in the monkey, shows that chemically determinable pyrimethamine was present in the sera of all monkeys for at least five days after the last dose of drug and in four of the six monkeys for seven days. Except for one period in one animal

TABLE 5

*Concentration of pyrimethamine in the serum of monkeys following oral administration**

Monkey number	Method of analysis	Mg. pyrimethamine per liter serum on day						
		0†	1†	2	3	4	6	8
6824	Chem.	0.90	1.90	1.65	0.60	0.40	0.25	0.15
	Micro.	0.56	1.22	1.07	0.53	0.21	0.08	0.07
6825	Chem.	1.10	1.90	1.75	0.80	0.45	0.20	0.10
	Micro.	0.84	1.53	1.59	0.51	0.18	0.09	0.00
6827	Chem.	0.85	1.65	1.45	0.55	0.35	0.20	0.05
	Micro.	0.47	2.02	1.27	0.32	0.22	0.09	0.00
6830	Chem.	0.65	1.85	1.65	0.55	0.55	0.10	0.00
	Micro.	0.47	1.57	1.49	0.73	0.18	0.12	0.06
6831	Chem.	0.80	2.35	2.55	0.80	0.45	0.30	0.20
	Micro.	0.45	1.68	1.91	0.54	0.34	0.13	0.05
6833	Chem.	0.90	1.55	1.35	0.70	0.35	0.20	0.15
	Micro.	0.50	0.79	1.10	0.42	0.30	0.09	0.00

* Doses of 5 mg./kg. were given on days 0 and 1.

† Serum samples on these days were obtained 2 hours after treatment.

the concentration of chemically measured pyrimethamine exceeded the amount which was found microbiologically. Thus there is no indication in the monkey that pyrimethamine is converted to a persisting metabolite with greater anti-folic activity than the parent compound. What should be emphasized is that in the monkey as in man pyrimethamine (or a substance with very similar chemical and microbiological properties) persists in the blood and tissues for relatively long periods. As will be pointed out in the subsequent discussion, this retention is quite sufficient to account for the prolonged protective effects of single doses of this drug against *P. vivax* infections in man and *P. cynomolgi* infections in the simian host.

The parallelism of the serum levels and excretion curves suggests that the materials measured by the two methods are similar. Further support for this conclusion was the finding that folic acid antagonists in the urine of human subjects and monkeys receiving pyrimethamine had chemical characteristics similar to the parent drug. Thus the anti-folic activity could be extracted from alkaline urine by ethylene dichloride (EDC) and reextracted by aqueous acid when the EDC phase was shaken with dilute HCl. Evidence for significant quantities of non-basic, EDC-insoluble folic acid antagonists was not found; in most instances treatment urine after EDC extraction produced no more growth inhibition in the *S. fecalis* system than control urine extracted in the same manner.

DISCUSSION

Ever since the early reports of Goodwin (1952) it has been generally believed that pyrimethamine is excreted rapidly following oral administration to the human subject. The prolonged suppressive or prophylactic effects of the com-

pound were attributed to its conversion to a hypothetical metabolite which was slowly excreted and more potent than the parent drug as a folic acid antagonist (and presumably more active as an antimalarial compound). The findings obtained in the present study demonstrate that in both man and rhesus monkey pyrimethamine is excreted slowly and persists in the body for a considerable period. In man the drug can be detected in urine for at least 11 days. The close parallelism between the concentrations of pyrimethamine as determined chemically and microbiologically (as folic acid antagonists) suggests strongly that the anti-folic acid activity is due primarily to the parent compound rather than to a metabolic derivative. The findings in the monkey are similar except that in this animal the data indicate that the parent drug is metabolized partially to substances indistinguishable from pyrimethamine chemically but less potent than the parent compound as antagonists of folic acid in the *S. fecalis* assay system.

These findings offer a simple explanation for the protracted suppressive activity of pyrimethamine against *P. vivax* infections in man and *P. cynomolgi* infections in the rhesus monkey. Thus Hernandez *et al.* (1953) demonstrated that a daily dose of only 0.25 mg. of pyrimethamine (one per cent of the usual weekly dose of 25 mg.) was required to suppress infections with *P. vivax* when administered at the time of infection. Data on urinary excretion of pyrimethamine obtained in the present study indicate that amounts in excess of this would be present in most individuals for at least eleven days after treatment and for considerably longer periods in some subjects. This prolonged excretion and hence presence of the pyrimidine would also explain the observation of Coatney *et al.* (1953) that single doses of 25 mg. of pyrimethamine can protect individuals from subsequent infection with trophozoites of *P. vivax* for periods of at least 17 days.

In the monkey, pyrimethamine is excreted somewhat more rapidly than in man. This finding agrees well with the observation of Schmidt and Fradkin (1956, to be published) that a single 1 mg. per kg. dose of pyrimethamine confers protection against subsequent infection with *P. cynomolgi* for about eight days. The minimum amount of pyrimethamine required to suppress *P. cynomolgi* is 0.04 mg. per kg. or about four per cent of the initial dose (1 mg. per kg.) employed. Since pyrimethamine is more rapidly excreted in the monkey and perhaps more extensively metabolized, the proportion of the initial dose still present in the body will probably drop to amounts below the minimum effective dose in about eight days.

Evaluation of the amounts of pyrimethamine required to suppress malarial infections and the amount probably present in the body at a given interval after administration would indicate, therefore, that it is unnecessary to postulate that pyrimethamine is converted to a more active (as folic acid antagonist) metabolic derivative in order to account for the protracted activity of the drug in human or simian malarias. These effects can be explained adequately by the observation that in both hosts the parent compound itself is retained in the body for rather long periods.

SUMMARY

After oral administration to human subjects, pyrimethamine could be detected chemically and microbiologically for seven days in the serum and for eleven days in the urine. Urinary excretion during the first five days accounted for about twelve per cent of the dose administered. The excretion patterns of materials measured by chemical and microbiological procedures were similar, an observation which speaks against conversion of the parent drug to a metabolite possessing greater anti-folic acid activity.

When administered orally to monkeys, pyrimethamine could be detected for at least five days in the serum and seven days in the urine. Urinary recovery in this period ranged from twenty to forty per cent by chemical determination and from ten to thirty per cent by microbiological assay. Chemically determinable pyrimethamine in both serum and urine was always greater than that measured microbiologically indicating that in the monkey pyrimethamine is converted to a metabolite which has less anti-folic activity than the parent compound.

The "anti-folic acid" activity in urine of human subjects and monkeys receiving pyrimethamine behaved chemically like the parent drug. Evidence for the presence of non-basic pyrimethamine metabolite(s) possessing a higher order of anti-folic activity than the parent compound was not obtained.

Correlation of these findings with earlier observations on the antimalarial activity of pyrimethamine suggests that the prolonged suppressive activity of this compound against infections with *Plasmodium vivax* in man and *P. cynomolgi* in the rhesus monkey is due to retention of the parent compound.

REFERENCES

Association of Official Agricultural Chemists, *Official Methods of Analysis of the Association of Official Agricultural Chemists*. 7th Edition, Washington, D. C., 1950, p. 784.

Coatney, G. R., Myatt, A. V., Hernandez, T., Jeffrey, G. M., and Cooper, W. C., 1953. Studies in human malaria. XXXII. The suppressive and therapeutic effects of pyrimethamine (Daraprim) against sporozoite-induced Chesson strain *vivax* malaria, *Am. J. Trop. Med. and Hyg.* **2**: 777–787.

Goodwin, L. G., 1952. Daraprim—clinical trials and pharmacology, *Tr. Roy. Soc. Trop. Med. and Hyg.* **46**: 485–495.

Hernandez, T., Myatt, A. V., Coatney, G. R., and Jeffrey, G. M., 1953. Studies in human malaria. XXXIV. Acquired resistance to pyrimethamine (Daraprim) by the Chesson strain of *Plasmodium vivax*, *Am. J. Trop. Med. and Hyg.* **2**: 797–804.

Hitchings, G. H., 1952. Daraprim as an antagonist of folic and folinic acids, *Tr. Roy. Soc. Trop. Med. and Hyg.* **46**: 467–473.

Schmidt, L. H., and Genther, C. S., 1953a. The antimalarial properties of 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (Daraprim), *J. Pharmacol. and Exper. Therap.* **107**: 61–91.

Schmidt, L. H., Hughes, H. B., and Schmidt, I. G., 1953b. The pharmacological properties 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (Daraprim), *J. Pharmacol. and Exper. Therap.* **107**: 92–130.

ELECTRON MICROSCOPE STUDIES OF SINDBIS VIRUS: A NEWLY RECOGNIZED ARTHROPOD-TRANSMITTED VIRUS

REGINALD L. REAGAN. FRANCES S. YANCEY, SING CHEN CHANG AND A. L. BRUECKNER

Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland

The virus was first encountered by Taylor and Hurlbut in August of 1952 in a group of 63 *Culex pipiens* or *Culex univittatus* mosquitoes captured by light traps in the Sindbis (Egypt) health district located several kilometers north of Cairo (Taylor *et al.*, 1955). Isolation had been made by inoculating a suspension of triturated mosquitoes into 3-day old mice. Serial filtration of the virus by passage from the larger to the succeedingly smaller pore size filters indicated an endpoint between pore sizes 95.8 and 79.4 mu. According to the method of Elford (1933), the size of the virus particles was calculated to lie between 48 and 40 mu which approximates the size of the equine encephalitis viruses. The virus has proved highly pathogenic to embryonating chicken eggs. Seven-day-old embryos exhibited generalized congestion, petechial hemorrhages of the integument and membranes and died within 24 and 72 hours after yolk sac inoculation with dilutions up to 10^{-7}. Embryos infected via the allantoic sac did not have allantoic fluid containing agglutinins for chicken erythrocytes (Taylor *et al.*, 1955).

PROCEDURE

The virus employed in this study was Ar339, M.G. 19431 Sindbis virus furnished by the American Type Culture Collection in Washington, D. C. (public health permit approved for work with this virus). The virus was received in the lyophilized state and was diluted with 2 ml. of physiological saline before initiation of the experiment. Twenty 7-day-old embryonating chicken eggs were inoculated aseptically into the yolk sac with 0.03 ml. of the reconstituted virus. Ten embryonating eggs were inoculated with sterile physiological saline into the yolk sac (0.03 ml.). Both sets of eggs were incubated at 37°C. and were candled twice daily. On the third day post inoculation, the embryos were generally congested and petechial hemorrhages of the membranes were present. The embryos were destroyed and allantoic-amniotic fluid was removed from the infected eggs after several hours chilling. The allantoic-amniotic fluid harvested from each infected egg was pooled and designated as group 1. Allantoic-amniotic fluid removed from the eggs inoculated with physiological saline was pooled and designated as control group 2. The two pooled suspensions of allantoic-amniotic fluid were centrifuged for five minutes in an angle centrifuge at 2,000 r.p.m. The supernatants were recovered from the specimens and filtered through type ST, size L3 Seitz filters. The filtrates were then subjected to centrifugation for three hours at 42,000 r.p.m. in the Spinco ultracentrifuge Model E under refrigeration. Temperature of the refrigerated outer jacket stayed constant at −10°C. during

centrifugation. After the three hour centrifugation period, the supernatants from the two specimens were discarded and the sediment from each was resuspended in 1 ml. of distilled water. Several drops of each suspension were placed on parlodion film supports which had been prepared seven days previously. After all excess fluid had been removed with fine capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/12 and examined under the R.C.A. electron microscope, type EMU. The balance of the infected specimen was inoculated (after addition of 500 units of penicillin and 250 mg. of streptomycin per ml. of fluid) into the yolk sac of ten 7-day-old embryonating chicken eggs. At the end of 48 hours, the embryonating

FIG. 1. Electron micrograph of normal control: showing no virus like particles. Shadowed with chromium at arc tangent 2/12. Magnification: ×40,000.

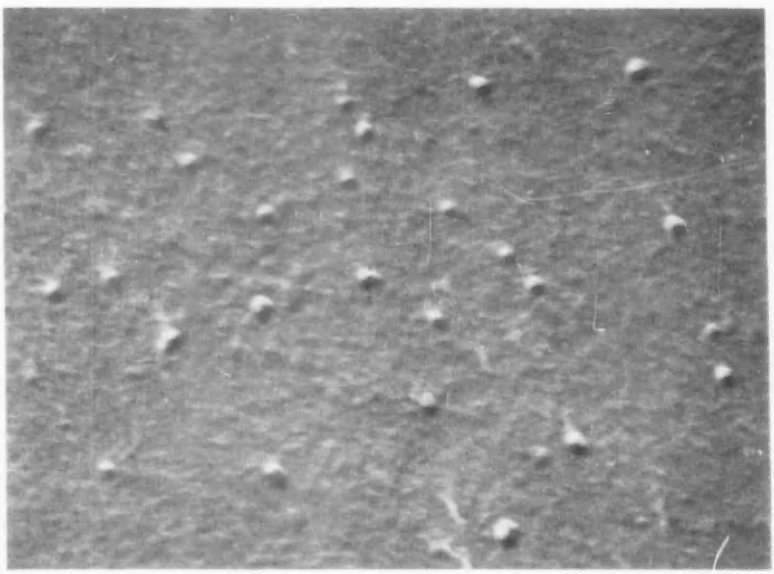


FIG. 2. Electron micrograph of the Sindbis virus. Shadowed with chromium at arc tangent 2/12. Magnification: ×100,000.

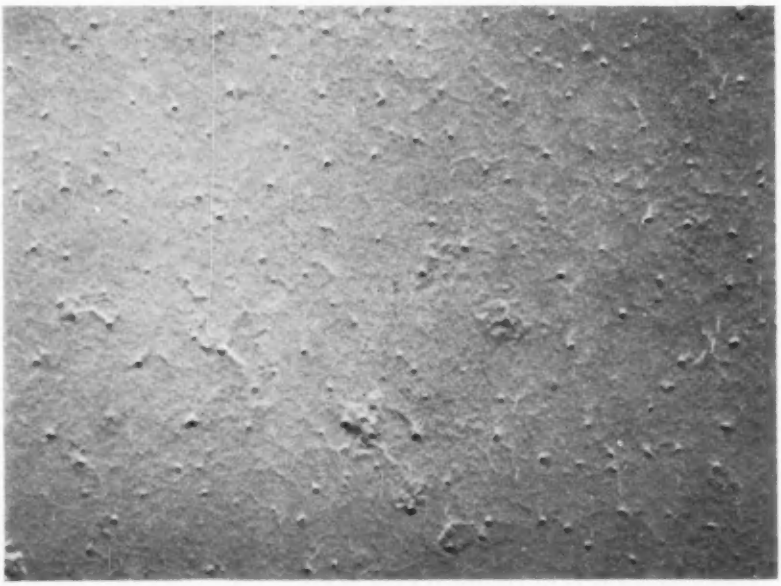


FIG. 3. Electron micrograph of the Sindbis virus. Shadowed with chromium at arc tangent 2/12. Magnification: ×40,000.

eggs were dead. By aseptic technic, a pool was made from the infected allantoic amniotic fluid and neutralizing tests were conducted in 7-day-old embryonating chicken eggs. The results confirmed the virus to be the Sindbis tropical virus.

RESULTS

Electron microscope examination of the concentrated suspension of allantoic-amniotic fluid from the embryonating chicken eggs injected in the yolk sac with physiological saline (group 2) revealed no virus-like particles to be present. Approximately 2,000 grid fields were examined. Figure 1 shows a representative field. Upon examination of the concentrated specimen from infected allantoic-amniotic fluid from eggs infected with the Sindbis virus, uniform virus-like particles were demonstrated as shown in Figures 2 and 3. These bodies measure 45 to 55 mu by direct measurement and were spherical in shape. This agrees with the size range of 40 to 48 mu reported by Taylor *et al.* (1955).

SUMMARY

Studies by electron microscopy of allantoic-amniotic fluid from embryonating chicken eggs infected with Sindbis virus show the virus to be spherical in shape with a diameter between 45 mu and 55 mu. These bodies could not be demonstrated in concentrated suspensions of normal filtered allantoic-amniotic fluid. Neutralization tests confirmed the virus to be Sinbis tropical virus.

REFERENCES

ELFORD, W. J., 1933. Principles of ultracentrifugation as applied to biolological studies, *Proc. Roy. Soc., London, Series B*, **112**: 384.

TAYLOR, R. M., HURLBUT, H. S., WORK, T. S., KINGSTON, J. R., AND FROTHINGHAM, T. E., 1955. Sindbis virus: a newly recognized arthropod-transmitted virus, *Am. J. Trop. Med. and Hyg.* **4**: 844.

WILLIAMS, R. C., AND WYCKOFF, R. W. G., 1946. Application of metallic shadowcasting to microscopy, *J. Appld. Physics* **17**: 23.

STUDIES ON DIARRHEAL DISEASES IN CENTRAL AMERICA[1]

I. Preliminary Findings on Cultural Surveys of Normal Population Groups in Guatemala

M. DORTHY BECK[2], J. ANTONIO MUÑOZ[3], and NEVIN S. SCRIMSHAW[4]

Instituto de Nutrición de Centro América y Panamá (INCAP) Guatemala, C. A.

High infant mortality is a serious and important problem in Guatemala. The extent to which the diarrheal diseases contribute to this high mortality is not known; specific etiological diagnoses have been few, and morbidity and mortality records have been sufficient only for general impressions. However, the published rates for infant deaths from diarrhea and enteritis are the highest in Central America (Pan Am. Sanit. Bur., 1954). Conclusions have been principally based upon cases admitted to the Guatemala General Hospital in Guatemala City. These cases have been mostly in children under 5 years of age from low income families and areas with inadequate sanitary facilities.

Although the type and distribution of the diarrhea strongly suggests shigellosis, isolations of *Shigella* have been reported only rarely by laboratories in Guatemala. However, statistics from other areas have also been found misleading when better techniques were used for the isolation and accurate identification of *Shigella* and *Salmonella* organisms (Watt *et al.*, 1953). The present study is a preliminary epidemiological and laboratory survey of the diarrheal diseases in Guatemala. It involved screening the general population for the common pathogens, *Shigella* and *Salmonella*. The presumption that *Shigella* is a major cause of diarrheal disease in this country was tested by comparing rates obtained in this survey with those from other areas in which prevalence of *Shigella* had been shown to be related to morbidity and mortality from diarrheal diseases.

PLAN OF STUDY AND MATERIAL SELECTED

Organization and operation. By the establishment of a bacteriological laboratory at the Instituto de Nutrición de Centro América y Panamá (INCAP), Guatemala City, it was possible to integrate the work of this study with existing INCAP field projects. With the cooperation of the mayor of each village, personnel of the Nutrition Field Unit of the Guatemalan Public Health Department ar-

[1] This investigation was supported by a grant-in-aid from the World Health Organization, and research grant No. A-981 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

[2] Pan American Sanitary Bureau Consultant to INCAP in Medical Bacteriology during the period October, 1955 to March, 1956.

[3] Chief of the Nutrition Field Unit, Guatemalan Public Health Department.

[4] Director, Instituto de Nutrición de Centro América y Panamá (INCAP). A cooperative Institute for the study of human nutrition, supported by the Governments of Costa Rica, El Salvador, Gautemala, Honduras, Nicaragua and Panama and administered by the Pan American Sanitary Bureau, Regional Office of the World Health Organization. INCAP Scientific Publication I-64.

ranged to have the mothers bring the pre-school children, and the teachers the school children to a central place[5]. This was either the school, a clinic maintained by the health department or the mayor's office. In Guatemala City, the children in day-care centers and pediatric clinics were included by arrangement with the physician in charge.

Scope of operations. Guatemala is a country which has great variation in climate and altitude; the survey was planned to include as many different localities as the duration of the consultant assignment of the senior author permitted, and to determine the prevalence of *Shigella* and *Salmonella* in these areas.

Table 1 presents background information on the 12 locations surveyed and includes data on the ethnic types, total population, and sanitary facilities of each. The localities varied from an elevation of less than 100 feet with a hot humid climate to an altitude of 8,399 feet with a cold dry climate.

The following descriptions of two different communities illustrate the major types of living conditions for persons included in the surveys;

Amititlán is a town with a population of approximately 10,000 inhabitants, 91 per cent of them reported as Ladino[6] and 9 per cent as Indian. The town is located near Lake Amatitlán at an elevation of 3,871 feet. There are 1,231 variously constructed houses, many of them with only dirt floors. There is a municipal water supply which serves only 192 of the houses; 828 have their own private wells and 211 use other supplies. These include lake water, community fountains, and various miscellaneous sources. Seventy per cent of the houses have either flush toilets or privies; the remainder, approximately 27 per cent, have neither. There is no information on about 3 per cent.

Santa María Cauqué is a small village with a population of about 700. It is located 20 miles from Guatemala City at an elevation of 6,493 feet. The population is 94 per cent Indian and 6 per cent Ladino. There are 206 houses in the village, mostly one-room adobe construction with dirt floors and no windows. There is no municipal water supply piped directly to the houses, but the village is furnished with water by 6 public fountains. Ten of the houses have open drains for sewers and the remainder have no provision for disposal of sewage.

Between the two extremes cited above, the rural communities selected showed various combinations of sanitary facilities, housing and population, but most of the living conditions were far below accepted minimum standards.

Children in Guatemala City were also surveyed in order to include a group with better community sanitary facilities. To obtain a good sized sample in a short time, specimens were collected from two sub-groups in Guatemala City.

[5] The most productive method of conducting these surveys elsewhere has been found to be visits and interviews (for details of family experience with diarrheal illness) with each household family to be included in the study. However, because of the short time available for this survey it was found necessary to omit house-to-house visiting and instead to bring the participants to a central place.

[6] Ladino is a Guatemalan term for a person of mixed origin who does not follow Mayan Indian customs of dress and modes of living. The term corresponds in part to that of Mestizo used for persons of mixed European and Indian ancestry, but includes Indians who have abandoned their own culture.

TABLE 1

Summary of information on twelve locations surveyed according to selected environmental factors, elevation and population Guatemala, 1955–1956

Location	Elevation (feet)	Population*			Total number houses	Water Supply†			Toilets†				Sewage System†				
		Total	Per cent			Municipal	Private wells	Others‡	Flush toilets	Privies	None	Unknown	Municipal	Cesspool	Open drain	None	Unknown
			Ladino	Indian													
Almolonga	6,808	3,735	3	97	534	1	74	459	—	440	94	—	—	1	23	510	—
Amatitlán	3,871	11,667	91	9	1,231	192	828	211	95	771	332	33	188	102	14	901	26
Coatepeque	1,594	8,449	90	10	1,262	418	611	233	184	820	154	104	244	21	893	—	104
La Esperanza	7,831	2,376	9	91	66	—	—	66	—	66	—	—	—	—	—	66	—
La Fragua	600	907	100	—	150	—	83	67	—	8	142	—	—	—	—	150	—
Guatemala	4,872	295,971	92	8	49,150	33,922	967	14,261	16,516	28,998	3,455	181	29,199	3,163	1,155	15,261	372
Masagua	367	575	92	8	123	17	37	69	1	43	56	23	—	2	98	—	23
Overo	85	538	100	—	89	—	75	14	1	30	54	4	—	1	84	—	4
San Mateo	8,399	840	—	100	60	—	—	60	—	29	31	—	—	—	—	60	—
Santa María Cauqué	6,493	800	6	94	206	—	—	206	—	10	196	—	—	—	10	196	—
Sumpango	5,974	5,780	9	91	889	23	1	865	2	416	470	1	13	17	94	751	14
Villa Nueva	4,298	7,429	90	10	604	111	1	492	6	252	345	1	69	18	1	515	1

* Sexto Censo General de Población, República de Guatemala, 1950. Dirección General de Estadística. Oficina Permanente del Censo, p. 87–89.

† By number of houses.

‡ Public fountains, streams, etc.

A. Those attending "Guarderías". These are child-care centers operated by semi-private welfare agencies with government support as a service to working mothers. The older children attend school while those in the younger group have organized play and kindergarten. They are well cared for, as the centers are adequately staffed and medical care is provided.

B. Those attending "Dispensarios". These are out-patient pediatric clinics operated by the municipality. Both sick and well children are brought to the clinics, which are staffed by physicians, nurses and attendants.

METHODS

The usual method (Watt *et al.*, 1953); Hardy and Watt, 1948; Watt and Hardy, 1945; Hollister *et al.*, 1955) for determining the prevalence rates for *Shigella* and *Salmonella* in general populations was followed in this study. This consists of obtaining rectal swab cultures from children 0–10 years of age. The rectal swabs taken in the field were planted immediately on an SS agar plate. The swab was then placed in a tube of the enrichment medium Selenite F.

Bacterial colonies which gave reactions consistent with *Salmonella* or *Shigella* in triple sugar iron agar were subjected to two coarse screening tests; (1) semisolid mannite agar for checking motility and acid production, and (2) urea agar. Following the screening tests, suspicious colonies were examined by additional biochemical tests, e.g., indol, citrate, etc., and by group agglutinations. Colonies tentatively identified as *Shigella* or *Salmonella* were sent to the Bacteriology Section, Division of Laboratories, California State Department of Public Health at Berkeley, for final definitive serological typing.[7]

RESULTS

During the period October, 1955, to March, 1956, a total of 2,342 rectal swab cultures were examined from children 0–10 years of age. In Table 2, the survey cultures are tabulated by the 12 locations and by number and per cent from which *Shigella* was isolated. An over-all prevalence rate of 7.5 per cent was observed; this varied from 3.7 to 16.2 per cent in the different communities. The differences noted could be related to the species of organisms responsible, and to socio-economic, sanitary, seasonal, or other variables. Some of these possible factors are discussed below.

Species of Shigella *isolated.* The distribution of the various species of *Shigella* isolated are presented in Table 3 and show a wide-spread distribution of nearly all types. The most important finding was the high percentage of Group A organisms. Two isolations were *S. dysenteriae 1* (Shiga type)[8] and 20 were *S. dysenteriae 2*. In the 1952–53 survey in California (Fresno County, 1953, unpublished) no organisms belonging to Group A were identified. Group D (*S. sonnei*) com-

[7] ***Shigella alkalescens*** was identified when found but has been excluded from all tabulations of ***Shigella*** prevalence.

[8] A third, *S. dysenteriae 1*, was isolated from a fatal case of diarrhea in a 23 year-old male. This is not included in the survey tabulations.

TABLE 2

Total survey cultures by geographic areas with prevalence rates for Shigella *Guatemala, 1955–1956*

Location	Total Cultures		
	Number	*Shigella*	
		Number	Per cent positive
Almolonga	69	3	4.3
Amatitlan	190	10	5.2
Coatepeque	217	15	6.9
Guatemala City	758	28	3.7
La Esperanza	109	8	6.4
La Fragua	138	15	10.8
Masagua	151	17	11.2
Overo	148	24	16.2
San Mateo	126	13	10.3
Santa María Cauqué	200	28	14.0
Sumpango	88	5	5.6
Villa Nueva	148	10	6.7
Total	2,342	176	7.5

prised 23.1 per cent of the isolations in Guatemala, while in the 1952–53 survey in Fresno County this group accounted for only 3.1 per cent of the isolations. However, in 1950, 13.3 per cent of isolations in California were Group D. The reason for the observed variations in groups is unknown and the predominance of a particular organism in any given area at any particular time is not explained.

Species of Salmonella *isolated.* Organisms belonging to the genus *Salmonella* were observed much less frequently in this study than were *Shigella.* Only thirteen isolations from 2,342 specimens were reported, an over-all rate of 0.55 per cent. Eight species were identified; four were *Salmonella newport*, three *Salmonella typhimurium*, and one each *Salmonella anatum*, *Salmonella denver*, *Salmonella derby*, *Salmonella manhattan*, *Salmonella panama*, and *Salmonella pharr.*

Since the observed prevalence rates for *Salmonella* were very low in the populations surveyed, the inference would be that this genus was of less importance in the diarrheal disease problem than *Shigella. Salmonella* prevalence rates were comparable to those found in general population surveys reported from the United States (Watt *et al.*, 1953).

Seasonal distribution. During the course of the survey a gradual decline in the prevalence rates for *Shigella* was noted from October to March. However, since most of the locations were surveyed during only one month, it is difficult to draw any conclusions as to the complete seasonal pattern of *Shigella* infection from these data.

Age distribution. As shown in Table 4, the prevalence rates for *Shigella* by age varied from a low of 2.7 per cent in the group under 1 year of age to a high of

TABLE 3

Species of the genus Shigella *isolated from rectal swab cultures Guatemala, 1955–1956*

Species	Total number	Percentage
Group A		
S. dysenteriae 1	2	1.1
S. dysenteriae 2	20*	11.3
Group B		
S. flexneri 1a	16	9.0
S. flexneri 1b	9	5.1
S. flexneri 2a	8	4.5
S. flexneri 3	30	17.0
S. flexneri 4a	10	5.6
S. flexneri 5	8	4.5
S. flexneri 6	29	16.5
Group C		
S. boydii 1	2	1.1
S. boydii 2	1	0.5
Group D		
S. sonnei	41*	23.1
Shigella—serotype 2110–55†	1	0.5
Total	177*	

* 1 person had 2 types.

† A new provisional type recognized by CDC in 1955.

11.8 per cent in the 1–2 year group. The rates decreased in each succeeding age group surveyed. The prevalence rates for *Shigella* for all ages surveyed in Guatemala are compared in Table 4 with those recorded from other areas where similar survey and cultural methods were used (Watt *et al.*, 1953; Watt and Hardy, 1945; Watt and Lindsay, 1948). The pattern was completely consistent with that obtained from these areas but considerable variation in magnitude was noted throughout.

In Guatemala the prevalance rates for each group were somewhat higher than those found in California in the 1950 survey and definitely higher than in Texas, South Georgia and California, 1952–1953. When the rates in 11 of the 12 communities surveyed exclusive of Guatemala City were compared, they were approximately of the same magnitude as those found in New Mexico, 1937–1938 (Hardy and Watt, 1948) when this area was experiencing extremely high morbidity and mortality from diarrheal diseases.

The low prevalence rates observed in the under-1-year age group in a number of surveys have aroused considerable discussion (Watt *et al.*, 1953; Fresno County, 1950, unpublished; Floyd *et al.*, 1956); similar low rates were obtained in Guatemala. The rates in individuals under six months of age were consistently lower than in the second six months. In Table 5, the prevalence rates for the group less than 1 year of age observed in several areas of the United States are compared with those found in Guatemala. This age group was further sub-

TABLE 4

Prevalence rates for Shigella *by age and year*
Guatemala, California, New Mexico, South Georgia and Texas

Age in years	Guatemala*	Guatemala exclusive of Guatemala City*	California camps*		New Mexico		South Georgia		Texas	
									No Fly control	Fly control
	1955–56	1955–56	1950	1952–53	1937–38	1948	1939	1949	1946–47	1946–47
					Total cultures					
Under 1	182	99	532	1,120	264	1,866	127	1,059	2,577	2,567
1	202	134	455	821	56	1,936	111	1,235	2,332	2,411
2–4	734	366	1,408	2,603	279	3,994	322	3,159	5,932	6,274
5–9	1,224	985	1,130	1,723	408	3,631	606	2,402	4,161	4,331
					Shigella positive					
Under 1	5	3	119	26	17	26	4	10	43	28
1	24	20	40	46	10	78	8	25	131	60
2–4	63	51	86	148	36	157	13	71	310	158
5–9	84	74	72	76	59	72	23	44	144	91
					Per cent positive					
Under 1	2.7	3.0	3.6	2.3	6.4	1.4	3.2	0.9	1.7	1.1
1	11.8	14.9	8.8	5.6	17.9	4.0	7.2	2.0	5.6	2.5
2–4	8.6	13.9	6.1	5.6	12.9	3.9	4.0	2.2	5.2	2.5
5–9	6.9	7.5	6.4	4.4	14.5	2.0	3.8	1.8	3.5	2.1

* Includes children 10 years of age.

divided into 3-month categories. In general, the rates increased with age, demonstrating a relationship between age and prevalence of *Shigella* infection which remained consistent in areas of both high and low endemicity.

Various theories have been advanced to explain this age distribution (Fresno County, 1950, unpublished; Floyd *et al.*, 1956). Increasing chance of exposure to infection with age has been given as one possible explanation. The results of an analysis of 1120 children under 1 year of age in California indicated that the opportunity for exposure did affect the prevalence rates. In households with *Shigella*-infected siblings the rates in children under 1 year were higher than in households with non-infected siblings, and lowest in households with no siblings. Other theories related to the presence of transplacental immunity, the significance of breast feeding in reducing exposure, improving nutritional status, and increasing immunity through the mother's milk, etc., or a combination of these factors, are not supported with sufficient data.

Prevalence rates in relation to selected variables. Watt and his associates (1953) have noted that shigellosis constitutes a major part of the diarrheal disease problem in areas characterized by low incomes, poor housing and inadequate sanitary

TABLE 5

Prevalence rates for Shigella *in children under 1 year of age by date of survey Guatemala, California, Texas, South Georgia and New Mexico*

Months of age	Total all areas	Guatemala	California		Texas	South Georgia	New Mexico
		1955–56	1950	1952–53	1946–48	1949	1948
Total cultures							
Total under 1 year	7,837	182	1,032	1,120	2,577	1,060	1,866
<6 months	3,676	65	511	532	1,254	451	863
0–2 months	1,548	19	178	224	559	205	363
3–5 months	2,128	46	333	308	695	246	500
>6 months	4,161	117	521	588	1,323	609	1,003
6–8 months	2,130	66	295	318	682	265	504
9–11 months	2,031	51	226	270	641	344	499
***Shigella* positive**							
Total under 1 year	136	5	26	26	43	10	26
<6 months	31	0	8	7	9	1	6
0–2 months	11	0	1	2	3	0	5
3–5 months	20	0	7	5	6	1	1
>6 months	105	5	18	19	34	9	20
6–8 months	44	2	9	8	13	4	8
9–11 months	61	3	9	11	21	5	12
Per cent positive							
Total under 1 year	1.7	2.7	2.5	2.3	1.7	0.9	1.4
<6 months	0.8	0.0	1.5	1.3	0.7	0.2	0.7
0–2 months	0.7	0.0	0.6	1.0	0.5	0.0	1.4
3–5 months	0.9	0.0	2.1	1.6	0.9	0.4	0.2
>6 months	2.5	4.2	3.4	3.2	2.5	1.4	1.9
6–8 months	2.0	1.5	3.1	2.5	1.9	1.5	1.6
9–11 months	3.0	5.8	4.0	4.0	3.3	1.5	2.4

facilities. In the present preliminary study the general information obtained on some of these variables is summarized in Table 1. Although the relationship of certain variables to *Shigella* prevalence rates was examined by application of the general observations to a proportion of the population, namely those 0–10 years of age, the environmental observations apply to the community as a whole.

Data from the 12 communities surveyed were grouped according to toilet facilities, availability of water supply, sewage disposal system, ethnic type and elevations. The findings are presented in Table 6. The prevalence rates were higher in each instance where the sanitary facilities were poor or lacking, where there was no closed sewage system or where promiscuous defecation was practiced. The Indian group had a higher prevalence rate than the Ladino group. This difference is probably not due to host susceptibility but rather to chances for exposure to infection. The Ladinos have somewhat better housing, more

TABLE 6

Prevalence rates for Shigella *observed in 12 locations grouped according to selected variables*
Guatemala, 1955–1956

Variables	Number of cultures	*Shigella*	
		Number	Per cent positive
Toilet facilities			
Privies and flush toilets in 50 per cent or over of houses	1,343	64	4.8
Under 50 per cent of houses	999	112	11.2
Water supply			
Private wells and municipal supply in 50 per cent or over of houses	1,451	92	6.3
community supply in over 50 per cent of houses	891	84	9.4
Sewage system			
Mixed—municipal, cesspools, open drains and none	1,401	68	4.8
Open drains and none	941	108	11.4
Ethnic type of population			
50 per cent or over, Ladino	1,750	119	6.8
50 per cent or over, Indian	592	57	9.6
Elevation			
Under 1,000 feet	437	56	12.8
1,000–2,999 feet	217	15	6.9
3,000–4,999 feet	1,096	48	4.5
5,000 feet and over	592	57	9.6

privies, and more private water supplies. It can be noted, however, that the observed prevalence rate for the predominantly Ladino community is close to the over-all rate of 7.5 per cent and high when compared with the results of most surveys done in the United States. Although the rates were high at the low elevations where the climate was hot and humid, the correlation with altitude was not at all consistent, and again the important factors were availability of water and better community facilities at whatever altitude.

Of the 12 communities surveyed (Table 1) Guatemala City, Coatepeque and Amatitlán possess more and better facilities in comparison to the other nine. On the basis of approximately equal numbers of cultures the prevalence rate for *Shigella* was found to be 4.4 per cent in the three larger communities with better sanitation, while in the remainder it averaged 10.4 per cent.

In general, the results obtained in this preliminary study fit the pattern observed in the other areas studied by these methods, i.e., when sanitary facilities are poor or lacking and when water is less available for personal use, the prevalence rates for *Shigella* are definitely higher (Hollister *et al.*, 1955; Stewart *et al.*, 1955).

SUMMARY

This preliminary study was designed to determine the prevalence of *Salmonella* and *Shigella* in human population groups without reference to the presence or

absence of frank diarrheal disease. Twelve communities in Guatemala were selected for study, and children 0–10 years of age examined by means of rectal swabs. Data on the external environment were obtained by observation and by compilation of locally available statistics.

The high prevalence rates for *Shigella* were shown to be comparable with rates obtained in selected specific areas of the United States during periods when *Shigella* infection was determined to be the major cause of diarrheal disease deaths. Although not all of the diarrheal problems in Guatemala are related to shigellosis, the data strongly suggest that organisms of the genus *Shigella* cause the major portion of diarrheal diseases in the communities studied in Guatemala and that diarrheal diseases caused by *Shigella* represent a major public health problem in the country.

The high rates in Guatemala, as in the United States, were associated with the lack of sanitary facilities, with poor housing, with limited water supply and with poor personal hygiene.

REFERENCES

Floyd, T. M., Higgins, A. R., and Kader, M. A., 1956. Studies in shigellosis. V. The relationship of age to the incidence of *Shigella* infections in Egyptian children, with special reference to shigellosis in the newborn and in infants in the first six months of life, *Amer. J. Trop. Med. and Hyg.* **5**: 119–130.

Hardy, A. V., and Watt, James, 1948. Studies of the acute diarrheal diseases. *XVIII. Epidemiology, Publ. Hlth. Rep.* **63**: 363–378.

Hollister Jr., A. C., Beck, M. D., Gittelsohn, A. M., and Hemphill, E. C., 1955. Influence of water availability on *Shigella* prevalence in children of farm labor families, *Amer. J. Publ. Hlth.* **45**: 354–362.

Pan American Sanitary Bureau Conference. XIV, Santiago, Chile, October, 1954. Summary of reports of the Member States, 1950–1953. Pan American Sanitary Bureau.

Sexto Censo General de Población, República de Guatemala, 1950. Dirección General de Estadística. Oficina Permanente del Censo, p. 87–89.

Stewart, W. H., McCabe, Jr., L. J. Hemphill, E. C., and De Capito, T., 1955. IV. Diarrheal disease control studies. The relationship of certain environmental factors to the prevalence of *Shigella* infection, *Amer. J. Trop. Med. and Hyg.* **4**: 718–724.

Watt, James, and Hardy, A. V., 1945. Studies of the acute diarrheal diseases. XIII. Cultural surveys of normal population groups, *Publ. Hlth. Rep.* **60**: 261–273.

Watt, James, Hollister Jr., A. C., Beck, M. D., and Hemphill, E. C., 1953. Diarrheal diseases in Fresno County, California, *Am. J. Publ. Hlth.* **43**: 728–741.

Watt, James, and Lindsay, D. R., 1948. Diarrheal disease control studies. I. Effect of fly control in a high morbidity area, *Publ. Hlth. Rep.* **63**: 1319–1334.

THE RELAPSE STATE IN TYPHOID FEVER TREATED WITH CHLORAMPHENICOL

KENNETH C. WATSON

Department of Pathology, Medical School, University of Natal, Durban, Natal, South Africa

Following the introduction of chloramphenicol in the treatment of typhoid fever it soon became apparent that, in spite of improvement in the clinical state which was usually manifest, there was no corresponding improvement in the relapse rate. Perreau and Bayle (1949) treated a series of 54 patients with a 13 per cent incidence of relapse. Medina *et al.* (1950) recorded 18 per cent of relapses in a series of 61 treated patients. Rankin and Grimble (1950) had no less than four relapses in a small series of nine patients treated. Several authors have compared the incidence of relapse in patients treated with chloramphenicol for varying periods of time and with varying dosages. Smadel, Woodward and Bailey (1949), for example, treated 44 patients with a loading dose of 3–4 gm. followed by 1–3 gm. daily. In thirteen patients where treatment was continued for eight days or less there were seven relapses. In the remaining 31 patients, treated for varying periods of 9–23 days, there were no relapses. The authors however, did not attempt to offer any explanation for these results. El Ramli (1953) also found a marked difference in the relapse incidence depending on the duration of chloramphenicol therapy, dated from the day the patient became afebrile. He divided a series of 398 cases into three groups. In the first group treatment was continued for from 0–3 days and the relapse rate was 26.3 per cent. In the second group treatment was given for seven days (after a rest period of seven days from the day of becoming afebrile). In this group the relapse rate was 13.8 per cent. The third group was treated for twelve days from the day of becoming afebrile with a relapse rate of only 3.9 per cent. Again, no explanation was offered for these differing results. Boquien *et al.* (1949) treated one group of 60 patients with chloramphenicol and compared the incidence of relapse with that of a second group of 60 patients none of whom received antibiotic therapy. The relapse rate in the former group was 11.7 per cent as compared with 6.7 per cent in the latter. El Ramli (1950a) recorded a relapse rate of 27.5 per cent in 200 patients treated with chloramphenicol. This compares with a rate of 13.9 per cent in 122 patients who did not receive any antibiotic (El Ramli, 1950b).

Further experience since these early reports has shown clearly that the incidence of relapse in typhoid fever treated with chloramphenicol depends on the duration of therapy and not to any extent on the actual dosage employed. Large loading doses for example do not reduce the relapse rate, and courses of therapy of less than seven days duration are usually associated with very high relapse rates. These observations of the effect of inadequate treatment raise a number of interesting points with regard to drug-host and drug-parasite relationships in typhoid fever. Since the introduction of chloramphenicol in the treatment of this disease its failure to reduce the incidence of relapse, the incidence of complications and the carrier rate have been disappointing features of this antibiotic.

During the past three years we have treated some 300 sporadic cases of typhoid fever. In each patient the course of chloramphenicol was continued for 10–15 days from the day of becoming afebrile, usually in a dosage of 250 mgm. every four to six hours. Our relapse rate in this series has been 6.0 per cent, which compares favorably with other reported series of adequately treated cases and also with the general experience of relapse rates in the pre-antibiotic days. We have been particularly interested in the antibody levels associated with the relapse phase because of the effect which the antibiotic may have in depressing the immune response. In all our relapse cases however, the level of somatic O antibody has been 1/640 or greater, at the time of relapse. We have not routinely determined flagellar antibody levels since this type of antibody has little protective power. Our experience is common to that of other workers, namely, that the relapse phase may occur at a time when the antibody response is maximal. The fact that the addition of a further suppressive agent in the form of chloramphenicol may, in certain dosages, increase the relapse rate rather than decrease it, has suggested to us a possible explanation of the drug-host relationship in the relapse phase.

NORMAL MODE OF PROTECTION AND OCCURRENCE OF RELAPSE IN TYPHOID FEVER

It is usually believed that humoral mechanisms are of the greatest importance in clearing the blood stream in typhoid fever and in bringing about clinical cure. In particular, somatic antibody is of more importance than flagellar antibody. Zinsser *et al.* (1944) suggest that tissue immunity may be increased as a result of infection but this concept is one that has received practically no support in recent years. The role of phagocytosis is even more difficult to evaluate. It is certain that phagocytosis of sensitized organisms takes place during the illness, but it is not so certain what percentage of such ingested forms is killed off by the phagocytic cells. In typhoid fever, as in tuberculosis and brucellosis, intracellular forms may remain viable and may in fact proliferate in their intracellular location (Adams, 1939). Goodpasture (1937) and Adams (1939) have demonstrated the organisms in plasma cells and lymphoid tissue in the intestine. These intracellular forms are probably the main reservoir responsible for initiation of the relapse phase and are probably widely distributed in reticulo-endothelial tissue.

It has been postulated that these tissue forms of *Salmonella typhi* are insusceptible to the action of antibiotic and antibody, because they may exist in a state of lowered metabolic activity. This theory is difficult of direct proof but it seems more probable that the failure of antibiotic and antibody to affect their viability is entirely dependent on the intracellular location of the organisms. Rous and Jones (1916) demonstrated protection of phagocytozed forms of *S. typhi* from the effect of immune antibody. Similarly, Magoffin and Spink (1951) have shown protection of intracellular forms of brucella from the action of streptomycin. Our own findings with *S. typhi* have been similar (Watson, 1955a). Smith *et al.* (1949) have shown that the cell wall appears to provide an effective barrier to the passage of chloramphenicol into the cell. Similarly, culture of various organs

from patients dying of typhoid fever has resulted in the isolation of *S. typhi* in spite of the administration of chloramphenicol during life (Reilly *et al.*, 1950; Marmion, 1952; Watson, 1954). It seems probable that in some patients release of these tissue forms into the blood stream may occur in sufficient amount to overcome the effect of circulating antibody and give rise to the clinical state of relapse.

The reinvasion of the blood stream, as mentioned above, almost certainly takes place at frequent intervals during the course of illness. This is borne out by our own observations detailed in Table 1. Since chloramphenicol has a definite but slow bactericidal action (Hobby *et al.*, 1950; Watson, 1955b) one would expect that the peripheral blood would be sterile in a matter of twenty-four to thirty-six hours after the first dose. Such indeed has been the experience of most workers. Cook and Marmion (1949) lay particular stress on this point. However, in our experience it has frequently been possible to isolate *S. typhi* from the blood stream, particularly from blood clot, in spite of simultaneous treatment with chloramphenicol. Table 1 shows such isolation in a series of twenty-two patients.

The results in Table 1 show that often, in spite of many days treatment with chloramphenicol, viable forms may still be present in the blood. The findings support our contention that there is probably frequent release of tissue forms of the organism into the blood stream.

TABLE 1

Isolation of Salmonella typhi *from the blood of patients during administration of chloramphenicol*

Case No.	No. of days treatment before specimen taken	Last dose before specimen taken (mgm.)	Total dosage (gm.)
1	10	250	10
2	2	500	4
3	1	500	2
4	2	250	2
5	1	500	2
6	1	500	1
7	8	250	8
8	2	500	2
9	2	500	3
10	11	500	22
11	2	500	2
12	2	250	1.5
13	2	250	3
14	2	250	3
15	3	250	4
16	1	250	1.5
17	2	250	3
18	2	500	2.5
19	2	250	2
20	2	250	2
21	12	250	15
22	17	250	17

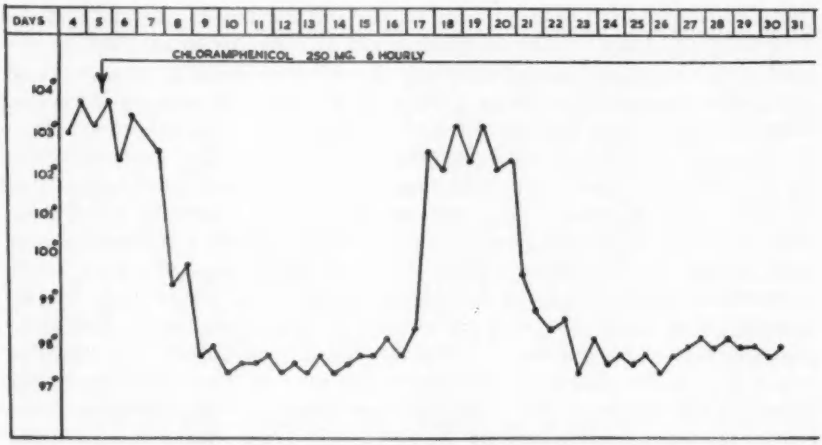


FIG. 1. Relapse during administration of chloramphenicol

In those patients where positive cultures were obtained in this way, after about the fourth day of treatment with antibiotic, there was usually great improvement in the clinical state and most of them were afebrile at the time of sampling the blood. However, in one patient clinical relapse occurred during the time that chloramphenicol was being given (Figure 1). In this patient there was presumably sufficient reinvasion of the blood stream to overcome the effect of both the antibiotic and immune antibody.

DISCUSSION

The very striking differences in the relapse rates of patients with typhoid fever treated with short term and with long term courses, from the day of defervescence, seem to indicate that chloramphenicol has some effect on the immune response. Typical of these differences are those quoted by El Ramli (1953). It is apparent that this effect is not always to the advantage of the patient.

Numerous studies have been conducted on the immune response in man and animals, as it is affected by the presence of antibiotic. Smadel (1954) has studied the effect of chloramphenicol on the developement of OXK agglutinins in scrub typhus and has shown that therapy begun early in the course of the disease will delay the production of antibody, although it did not affect the final antibody levels. Smadel concluded that the mode of action in this instance was a diminution in the amount of antigenic material available for antibody production rather than a failure of the antibody-forming mechanism to function properly in the presence of antibiotic. Similarly, Green *et al.* (1951) found no difference in antibody levels following T.A.B. vaccination in a series of patients treated with penicillin and in a control group. Stevens (1953) however, measured antibody production in rabbits by the method of following the rate of antigen removal. The antigen in this case was radio-iodinated bovine gamma globulin. He found that

penicillin, dihydrostreptomycin, aureomycin and terramycin all produced depression of antibody production which could only be due to an effect on the antibody forming mechanism. However, this response was not constant in all the rabbits used and some did not exhibit any depression of antibody formation. These findings require further confirmation. Other workers have been unable to demonstrate any effect on antibody production by antibiotics. Harrison (1946) for example, did not find any difference in the level of protective antibody or agglutinins against pneumococci, with and without the administration of penicillin. Marchal *et al.* (1949) found that streptomycin appeared to stimulate antibody production to typhoid vaccine, but that penicillin on the other hand, appeared to depress the antibody response. Jordan *et al.* (1950) could find no apparent alteration in the serological response in cases of pneumococcal pneumonia treated with penicillin, as compared with a control group. Wannamaker *et al.* (1951) on the other hand, have shown that early treatment of streptococcal infections with penicillin inhibits production of antistreptolysin. Similarly, Weinstein *et al.* (1949) and Daikos and Weinstein (1951) have shown that penicillin may inhibit production of antistreptokinase, antistreptolysin, anti-M and anti-C antibody. Smadel's findings (Smadel—1954) suggest that depression of antibody formation only takes place where treatment is begun in the early stages of infection. The majority of workers who have described antibody depression in the presence of antibiotic agree with Smadel's theory of inadequate antigenic material being available in the early stages of infection. However, it would seem that if Stevens's observations are correct, other factors may play a part.

With regard to the production of antibody in typhoid fever, our own experience would lead us to agree with Smadel (1954) that treatment begun in the first few days of illness will delay the production of antibody but will not affect the final levels. However, the great majority of our patients were admitted after the seventh day of illness and at this stage antibody production is not affected by the administration of chloramphenicol. In the cases of scrub typhus described by Smadel (1954), treatment on the third and sixth days of illness respectively were associated with relapse rates of 62 per cent and 37 per cent. There were no relapses in a series of patients treated on the eighth day of illness. The relapses in the first two groups could be correlated with the absence of sufficient antibody. The problem of the relapse in typhoid fever however, is an entirely different one since here the relapse phase takes place late in the disease at a time when high antibody levels can be demonstrated.

Any hypothesis regarding the effect of chloramphenicol on the relapse state in typhoid fever must take into account this factor, namely, that relapse takes place in spite of high antibody levels. There are three main points which require consideration. First, why is the relapse rate higher in patients given a short course of therapy than in those treated for a longer period of time? Second, why has there been no reduction in the incidence of relapse in patients treated with adequate doses of chloramphenicol compared with the untreated patients, and third, why should the patients inadequately treated with chloramphenicol have a very much higher relapse rate than the untreated patients?

With regard to the first group, if the theory of release of intracellular tissue forms of the organism is correct, as we believe, then the administration of chloramphenicol for a period of fourteen days or so will suppress the organisms as they are released into the blood stream. However, Figure 1 shows that this mechanism is not always successful. By the time the administration of the chloramphenicol is stopped, the concentration of tissue forms of the organism will have fallen to a level too low to release sufficient organisms into the blood stream to cause clinical relapse. This is because the majority of the tissue forms will have been disposed of by the normal phagocytic mechanisms, although as stressed above, many of the intracellular forms can remain viable. In those patients treated with a course of chloramphenicol lasting only three or four days however, the concentration of organisms in the tissues, when therapy is stopped, will be high enough to cause reinvasion of the blood stream and clinical relapse since there will have been less time available for their disposal by the phagocytic cells than in the previous group. The difference in the incidence of relapse with the two methods of therapy lies mainly, we believe, in the relative numbers of organisms potentially available in tissues for reinvasion of the blood stream at the time when the concentration of antibiotic in the blood is no longer effective.

The incidence of relapse in patients treated with adequate doses of chloramphenicol over a period of ten to fourteen days from the day of becoming afebrile, is practically identical with that of untreated cases. This failure of chloramphenicol to cause any material reduction in the relapse rate is, we believe, essentially due to the ability of a certain number of organisms to remain viable in the intracellular position. Since chloramphenicol has only a very slow bactericidal action on *S. typhi* there would be adequate time available for the organisms to gain their intracellular position before being affected by the antibiotic. Here, once again, relapse probably takes place by reinvasion of the blood stream once the effect of the chloramphenicol has worn off. What evidence there is suggests that the intracellular position protects the organisms from the action of circulating chloramphenicol. We have quoted the findings of Smith *et al.* (1949) in this connection, and are at present investigating the matter further.

With regard to the third group mentioned above, it has been a common finding that short term treatment of typhoid fever lasting only a few days is often followed by a very high incidence of relapse. This was particularly noticeable in the early days of chloramphenicol therapy when the drug was in short supply. No satisfactory explanation of these observations has so far been advanced. A number of workers have, as mentioned above, reported that chloramphenicol may inhibit antibody production and have related the high relapses rates to this factor. Such an explanation however, does not fit the observed facts since the relapse usually takes place seven to ten days after therapy has been discontinued when antibody levels may be very high. Some other effect on the host-parasite relationship must be involved. One might postulate that the antibiotic has some effect on antibody in such a way as to inhibit its protective power without affecting its agglutinating properties. This however, is improbable for two reasons. First, the chloramphenicol is excreted in a matter of thirty-six hours at the latest,

mostly in the urine in the form of an inactive nitro compound, and it is difficult to imagine any possible alteration in the spatial configuration of the antibody molecule once the antibiotic is no longer present. Second, it is also improbable that antibiotic could affect antibody being produced during the period from the time the antibiotic is stopped and the onset of the relapse phase.

A more rational hypothesis however, can be advanced to explain this high incidence of relapse with inadequate antibiotic therapy. A number of workers have shown that treatment of certain organisms with streptomycin or penicillin sensitizes the organisms to phagocytosis (Linz and Lecocq, 1953; Linz, 1953; Dragoni, 1953). Similarly, we believe that in typhoid fever the administration of chloramphenicol results in a more rapid rate of phagocytosis than in the untreated case. If the course of treatment is short then, when the effect of the antibiotic wears off, there will be a large reservoir of tissue forms of the organism able to reinvade the blood stream and cause clinical relapse. As treatment is continued however, the chance of relapse will diminish since death of some of the intracellular forms will be taking place during this time. It may be objected that if there is increased phagocytosis as the result of chloramphenicol therapy, this is secondary to some damaging effect on the organisms and that such organisms would be killed off by the phagocytic cells. Phagocytosis however, will remove the organism from any further action of the antibiotic, presumably with recovery of those forms which have not been greatly affected.

The concept of the existence of a number of foci of intracellular forms of *S. typhi* can also be advanced to explain the carrier state in this disease. It is a common observation that the chronic carrier may be unaffected by the removal of the gall bladder. The observations of Adams (1939), who has shown the presence of intracellular organisms in plasma cells of the Peyer's patches, would well fit the explanation that they are partly responsible for maintaining the carrier state.

The above hypothesis of the mode of relapse in the presence of chloramphenicol is difficult of direct proof. It does however, explain why relapse may occur at a time when the antibiotic is no longer present in the blood and tissue fluids and why it may occur in spite of high antibody levels.

SUMMARY

The incidence of relapse in typhoid fever treated with chloramphenicol is dependent on the period of time during which therapy is continued. Where this is less than about seven days very high relapse rates may be experienced. The relapse state is probably initiated by intracellular forms of *S. typhi* present in reticuloendothelial cells. It is suggested that chloramphenicol promotes phagocytosis of viable organisms and that this creates a reservoir of tissue forms of the organism which is responsible for the high relapse rates in those patients who are inadequately treated.

ACKNOWLEDGEMENT

I am indebted to Dr. R. E. Stevenson, Director Medical and Health Services, Natal, for permission to publish, and to Dr. W. Laurie, Senior Pathologist, Natal Provincial Administration, for helpful criticism.

REFERENCES

ADAMS, J. W., 1939. Intracellular bacilli in intestinal and mesenteric lesions of typhoid fever, *Am. J. Path.* **15:** 561–566.

BOQUIEN, Y., HERVOUET, D., AND HILLERITEAU, H., 1949. Résultats du traitement par la chloromycétine de soixante fièvres typhoides, *Bull. et Mém. Soc. Méd. de Hôp. de Paris* **32:** 1396–1401.

COOK, A. T., AND MARMION, D. E., 1949. Chloromycetin in the treatment of typhoid fever, *Lancet* **2:** 975–979.

DAIKOS, G. AND WEINSTEIN, L., 1951. Streptococcal bacteriostatic antibody in patients treated with penicillin. *Proc. Soc. Exper. Biol. & Med.* **78:** 160–163.

DRAGONI, G., 1953. Azione della streptomicina e della lipasi pancreatica sul mycobacterium tuberculosis nella fagocitosi in vitro, *Boll. Soc. Ital. Biol. Sper.* **29:** 651–654.

EL RAMLI, A. H., 1950a. Chloromycetin in treatment of enteric group of fevers, *J. Roy. Egypt Med. Ass.* **33:** 40–55.

EL RAMLI, A. H., 1950b. Chloramphenicol in typhoid fever, *Lancet* **1:** 618–620.

EL RAMLI, A. H., 1953. Chloramphenicol in typhoid and paratyphoid fevers: New lines of treatment, *Lancet* **1:** 927–929.

GOODPASTURE, E. W., 1937. Concerning pathogenesis of typhoid fever, *Am. J. Path.* **13:** 175–186.

GREEN, S., WOHL, M. G. AND WAIFE, S. O., 1951. The effect of penicillin on typhoid antibody production, *J. Infect. Dis.* **89:** 169–172.

HARRISON, P. E., 1946. Comparative effect of penicillin and sulphonamide drugs on immune response of rabbits to pneumococcus infection and relation of immunity to bacterial chemotherapy, *J. Infect. Dis.* **79:** 101–130.

HOBBY, G. L., LENERT, T. F. AND DOUGHERTY, N., 1950. Newer antibiotics: Factors influencing their antimicrobial activity, *Ann. New York Acad. Sci.* **53:** 27–37.

JORDAN, W. S., BADGER, C. F., AND DINGLE, J. H., 1950. Immunological studies of pneumococcal pneumonia in patients treated with penicillin, *J. Clin. Invest.* **29:** 161–168.

LINZ, R., 1953. Sur le mécanisme de l'action de la streptomycine: le cas de Mycobacterium tuberculosis, *Ann. Inst. Pasteur.* **85:** 295–307.

LINZ, R. AND LECOCQ, E., 1953. Pénicilline et de la streptomycine sur la phagocytose in vivo des bacteries, *Compt. Rend. Soc. de Biol.* **146:** 785–788.

MAGOFFIN, R. L. AND SPINK, W. W., 1951. The protection of intracellular brucella against streptomycin alone and in combination with other antibiotics. *J. Lab. Clin. Med.* **37:** 924–930.

MARCHAL, J. G., NICOT, R. AND FERY, H., 1949. Influence de la pénicilline et de la streptomycine sur la formation des anticorps au cours de la vaccination antityphoïdique chez le lapin, *Compt. Rend. Soc. de Biol.* **143:** 1369–1372.

MARMION, D. E., 1952. The treatment of typhoid fever with chloramphenicol. A clinical study of 330 cases of enteric fever treated in Egypt, *Trans. Roy. Soc. Trop. Med. & Hyg.* **46:** 619–638.

MEDINA, I., PAYNE, E. H., AND SHARP, E. A., 1950. Typhoid and chloromycetin—a study of minimum dosage, *Am. J. Trop. Med. & Hyg.* **53:** 119–124.

PERREAU. P. AND BAYLE, V., 1949. Le traitement de la fièvre typhoïde par la chloromycétine, à propos de 54 cas, *Bull. et Mém. Soc. Méd. de Hôp. de Paris* **65:** 1401–1406.

RANKIN, A. L. K. AND GRIMBLE, A. S., 1950. Treatment of typhoid fever with chloramphenicol, *Lancet* **1:** 615–618.

REILLY, J., COMPAGNON, A., TOURNIER, P., BASTIN, R., AND DU BUIT, H., 1950. Les accidents du traitement des fièvres typhoïdes par la chloromycétine; étude expérimentale et déductions thérapeutiques, *Ann. Med. Paris* **51:** 597–654.

ROUS, P. AND JONES, F. S., 1916. The protection of pathogenic microorganisms by living tissue cells, *J. Exper. Med.* **23:** 601–612.

SMADEL, J. E., 1954. Influence of antibiotics on immunologic responses in scrub typhus, *Am. J. Med.* **17:** 246–258.

SMADEL, J. E., WOODWARD, T. E., AND BAILEY, C. A., 1949. Relation of relapses in typhoid to duration of chloramphenicol therapy, *J. A. M. A.* **141:** 129.

SMITH, G. N., WORREL, C. S., AND SWANSON, A. L., 1949. Inhibition of bacterial esterases by chloramphenicol (chloromycetin), *J. Bact.* **58:** 803–809.

STEVENS, K. M., 1953. Effect of antibiotics upon the immune response, *J. Immunol.* **71:** 119–124.

WANNAMAKER, L. W., RAMMELKAMP, C. H., JR., DENNY, F. W., BRINK, W. R., HOUSE, H. B., HAHN, E. O., AND DINGLE, J. H., 1951. Prophylaxis of acute rheumatic fever by treatment of the preceding streptococcal infection with various amounts of depot penicillin, *Am. J. Med.* **10:** 673–695.

WATSON, K. C., 1954. Chloramphenicol in typhoid fever: A review of 110 cases, *Trans. Roy. Soc. Trop. Med. & Hyg.* **48:** 526–532.

WATSON, K. C., 1955. Effect of chloramphenicol on the isolation of *S. typhi* from the blood stream, *J. Clin. Path.* **8:** 55–57.

WATSON, K. C., 1955b. In vitro activity of chloramphenicol on *Salmonella typhi*, *J. Lab. Clin. Med.* **45:** 97–101.

WEINSTEIN, L., BACHRACH, L., AND PERRIN, T. S., 1949. Studies of the influence of penicillin on the immune reactions in streptococcal pharyngitis, *J. Clin. Invest.* **28:** 817–818.

ZINSSER, H., ENDERS, J. F., AND FOTHERGILL, L. D., 1944. *Immunity: Principles and Application in Medicine* and *Public Health*. MacMillan, New York.

ECOLOGY OF RINGWORM FUNGI ON COMMENSAL RATS FROM RURAL PREMISES IN SOUTHWESTERN GEORGIA

WILLIAM W. SMITH, ROBERT W. MENGES AND LUCILLE K. GEORG

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Newton and Atlanta, Georgia

A cooperative investigation of the occurrence in rodents of pathogenic fungi infectious to man was undertaken at the Newton Field Station, Newton, Georgia, in August 1954, as one part of the study of the diseases transmissible to man for which other mammals are reservoirs.

Two species of fungi now known to occur frequently in southwestern Georgia are *Trichophyton mentagrophytes* and *Microsporum gypseum*. Reviewing the literature, Ajello (1953), and Menges and Georg (1954) noted that both these micro-organisms have been isolated from man, cats, dogs, horses, mice, and monkeys; the former species has also been cultured from cattle, chinchilla, fox, guinea pig, muskrat, opossum, rabbit, squirrel, and kangaroo; and the latter from a chicken and a tiger. It was estimated by Georg that 70 to 80 per cent of cases of human ringworm in rural areas were of animal origin. Such cases are presumed to be due to contact of man with infected animals or infected material. This paper reports the occurrence of these organisms on commensal rats taken from rural premises in southwestern Georgia.

METHODS

In connection with ecological studies of typhus eradication and leptospirosis, commensal Norway and roof rats (*Rattus norvegicus* and *R. rattus* subsp.) were collected at rural premises located in Grady, Thomas, Mitchell, and Baker Counties. Premises were chosen at random for trapping throughout Mitchell and Thomas Counties, and in Grady County except for the southeastern quarter where all the premises were surveyed for rat infestation. In Baker County trapping was done on one place only. Rats were trapped in unbaited steel traps placed in rat runs inside or near farm buildings. The rats alive at the time of the morning collection were bagged and transported to the Field Station laboratory where hairs were plucked with sterile forceps. The hairs were taken from the rat's muzzle, between the ears, near any apparent skin lesions, and from the base of the tail. These hairs were placed in envelopes and dispatched to the Communicable Disease Center Mycology Laboratory at Chamblee, Georgia. There they were tested by microscopic examination, fluorescence, and culture methods described by Georg (1954).

Trap records indicated the number of traps set at each of the premises, the number of rats caught (dead, living, and rat feet left in traps), and the number of nights that traps were set. Ordinarily trapping was done on premises for two or three consecutive nights. The frequency distribution of thirty premises chosen at random for trapping showed that approximately equal numbers of the premises

fell into three groups based upon percentages of trapping success. One-third of the premises were in the 0 to 5.9 per cent trap success group and were considered lightly infested; one-third were in the 6.0 to 10.9 per cent group and were considered to have medium infestations; the remainder showed more than 10.9 per cent trap success (range 11.8 to 27.2 per cent) and were considered heavily infested.

Soil samples consisting of approximately one-half pint of topsoil were collected from rat ranges, but not necessarily from rat runs at premises where rats with ringworm had been collected. These were forwarded to the Mycology Laboratory where they were cultured by using the hair baiting technique (Ajello, 1953).

RESULTS

A total of 434 commensal rats (84 roof rats, 350 Norway rats) were trapped from 162 farm premises located in four counties of southwestern Georgia. Microscopic examinations and fluorescence tests of the rat hairs submitted for testing failed to show the presence of ringworm fungi. Therefore, all positive tests reported here are the result of culture methods. Table 1 summarizes this study with reference to species and numbers of rats tested and the number with reference to species and number of rats tested and the number and percentage positive in each of four counties. It is to be noted that only Norway rats were obtained for testing in Mitchell, Thomas, and Baker Counties. None of the rats obtained in Mitchell County were positive. In the remaining counties, all positives except the two from Baker County were the result of *T. mentagrophytes* infections. *M. gypseum* was isolated from the hairs of the two Baker County rats. The prevalence of *T. mentagrophytes* is indicated to be approximately 8 per cent in the commensal rats of the counties where it was found present (20 positive from 249 tested). The prevalence in Norway rats, 9.1 per cent (15 positive from 165 tested) is slightly higher than that for roof rats, 7.1 per cent (5 positive from 84 tested), but not significantly so by the chi-square test.

TABLE 1

Prevalence of ringworm fungi on commensal rats trapped from rural premises in Southwestern Georgia, Aug. 25, 1954 to Oct. 5, 1955

	R. Norvegicus			*R. Rattus* Subsp.		
County	No. tested	No. positive	% positive	No. tested	No. positive	% positive
Mitchell	150	0	0.0	0	0	0
Grady	104	10*	9.6	84	5*	7.1
Thomas	61	5*	8.2	0	0	0
Baker	35	2†	5.7	0	0	0
Total	350	17	4.9	84	5	7.1

* *Trichophyton mentagrophytes.*

† *Microsporum gypseum.*

TABLE 2

Rat density as related to percentages of rural premises with ringworm-positive rats in Southwestern Georgia; Aug. 25, 1954 to Oct. 5, 1955

Location	Heavy			Medium			Light			County Totals		
	No. premises examined	No. positive	% positive	No. premises examined	No. positive	% positive	No. premises examined	No. positive	% positive	No. premises examined	No. premises with positive rats	% premises with positive rats
Mitchell County	18	0	0.0	13	0	0.0	22	0	0.0	53	0	0.0
Grady County (Typhus eradication area)	23	8	34.8	18	2	11.1	26	1	3.8	67	11	16.4
Thomas and Grady Counties (untreated)	17	6	35.3	15	2	13.3	9	1	11.1	41	9	21.9
Baker County	1	1	100.0	0	0	0.0	0	0	0.0	1	1	100.0
Total	59	15	25.4	46	4	8.7	57	2	3.5	162	21	13.0

In Table 2, the relationship between the degree of rat infestation of premises and the prevalence of ringworm in rats on the premises is explored. The regions where the trapping was done are listed in chronological order. Trapping in Mitchell County was accomplished from August 25 to November 11, 1954; in the southeastern quarter of Grady County (typhus eradication area of Mohr and Smith, 1956) from February 8 to June 8, 1955; and in the untreated region of Grady and Thomas Counties from June 15 through September 23, 1955. All rats from Baker County were obtained during June, 1955. No rats with ringworm were found in Mitchell County. This county is not known to harbor any roof rats. Baker County is also believed to be free of roof rats.

Where ringworm caused by *T. mentagrophytes* was found on rats in a county region, more premises which supported heavy rat infestations had rats with ringworm (34.8 and 35.3 per cent positive) than those with light infestations (3.8 and 11.1 per cent positive). There is no significant difference between the percentages of premises with rats positive for ringworm in the Grady County typhus eradication area (16.4 per cent positive) and those from the untreated portions of Grady and Thomas Counties (21.9 per cent positive) as indicated by the chi-square test. Since trapping was done on only one property in Baker County, no conclusions may be reached except that ringworm was present on two Norway rats from the heavily rat-infested premises. The fungus isolated was *M. gypseum* and this was the only instance in which it was found on rats during the study. In all, thirteen of the 162 premises from which rats were tested had rats with ringworm infections.

In Table 3, only premises where rats with ringworm were found are considered in the attempt to relate rat density to the percentage of rats infected. Of 16 premises where ringworm-infected rats were trapped, 10 were computed to have heavy infestations. Seventy-nine rats were tested from these farms, 15 (19 per cent) being found positive. Seven of 13 (53.8 per cent) rats from farms with

TABLE 3

Rat density as related to incidence of ringworm in rats from rural premises in Southwestern Georgia; Aug. 25, 1954 to Oct. 5, 1955

	Degree of Rat Infestation of Premises	
	Heavy	Medium or light
No. premises	10	6
No. rats tested	79	13
No. rats positive	15	7
% rats positive	19.0	53.8

TABLE 4

Occurrence of Microsporum gypseum *in soil samples from rural premises harboring ringworm infested commensal rats in Southwestern Georgia; August, 1955*

Location	No. Premises Sampled	No. Samples	% Samples Positive
Grady County	5	20	55.0
Baker County	1	8	62.5
Total	6	28	57.2

lighter rat infestations were positive. An inverse relationship appears to be indicated by these limited data.

Table 4 shows the results of soil sampling. As yet, a relatively small number of soil samples have been tested. These were obtained from Grady and Baker Counties, and were taken from rat ranges but not altogether from rat runs or trails. *M. gypseum* was the only pathogenic fungus isolated. It was cultured from over half of the samples. As this fungus is known to be saprophytic (Ajello, 1953) as well as dermatophytic, its presence in the soil is not unexpected.

DISCUSSION

There is no adequate explanation for the absence or failure to demonstrate the presence of ringworm in rats taken from Mitchell County. This is particularly apparent when it is considered that rats were collected at random throughout the rural regions of the county. Factors which possibly might account for its absence are the location of Mitchell (and Baker) County to the north of the Grady and Thomas Counties region where the fungus *T. mentagrophytes* was found in rats; the fact that the two more northern counties have no roof rat colonies; and the trapping of the Mitchell County region at an earlier period (fall, 1954) than the other regions (1955).

It is logical to expect that premises with heavy rat populations would be more likely to have some rats with ringworm if the fungus were present. This is shown to be true in Table 2 where 34.8 and 35.3 per cent of premises with heavy rat infestations had rats with ringworm in Thomas and Grady Counties as contrasted to 11.1 and 13.3 per cent for premises with medium infestations, and 3.8 and 11.1 per cent for premises with light infestations. It would seem to follow

that premises with heavy rat infestations should have a higher percentage of rats infected than those premises with medium or light rat infestations. This reasoning is not substantiated by the data of Table 3 which indicate that an inverse relationship exists between rat density and percentages of rats with ringworm. It is possible that inadequate sampling may account for this discrepancy since few rats may be obtained for testing from lightly infested farms.

Soil samples showed the presence of *M. gypseum* only. None of the rats from Grady or Thomas Counties were infected with this fungus though it was readily recovered from over half of the soil samples taken from five premises known to have harbored rats with *T. mentagrophytes* infections. It appeared to be significant only in Baker County where it was isolated from 5 out of 8 soil samples and 2 out of 35 rats taken from one farm.

SUMMARY

Two of the fungi, *Trichophyton mentagrophytes* and *Microsporum gypseum* which cause ringworm in man infect many domestic and wild animals in nature. Most of the human cases of *T. mentagrophytes* in rural areas are presumed to be of animal origin, but *M. gypseum* infections are believed to be acquired more often from soil contacts.

Commensal rats were collected from rural premises in four counties of southwestern Georgia and their hairs were tested for ringworm. Approximately 5 per cent of the 350 Norway rats and 7 per cent of the 84 roof rats trapped had ringworm infections as shown by culture methods. Only Norway rats were caught in Mitchell County and none of 150 specimens were found infected. Rats positive to *T. mentagrophytes* came only from Grady and Thomas Counties. Two Norway rats from the 35 trapped in Baker County showed the presence of *M. gypseum*. The degree of rat infestation of premises as indicated by success in trapping was directly related to the percentages of premises with ringworm-positive rats. An inverse relationship between the degrees of rat infestation of premises and percentages of rats infected by ringworm was indicated by the limited data considered.

Twenty-eight soil samples taken from rat ranges on six premises where ringworm-positive rats were trapped showed the presence of *M. gypseum* only, over half of the samples containing this primarily saprophytic fungus.

REFERENCES

AJELLO, L., 1953. The dermatophyte, *Microsporum gypseum*, as a saprophyte and parasite, *J. Invest. Derm.* **21**: 157–171.

GEORG, L. K., 1954. The diagnosis of ringworm in animals, *Vet. Med.* **49**: 157–166.

MENGES, R. W., AND GEORG, L. K., 1955. Animal ringworm study, *Vet. Med.* **50**: 293–297.

MOHR, C. O., AND SMITH, W. W., 1956. A preliminary attempt to eradicate murine typhus from a rural endemic area, *Bull. WHO.* (Manuscript).

HUMAN ISOSPORIASIS: A REPORT OF THREE CASES WITH NECROPSY FINDINGS OF ONE CASE

B. H. WEBSTER

Human isosporiasis remains a rare and poorly understood disease even though about three hundred cases are described in the medical literature (Wells and Bucki, 1953). Some of the cases reported are superimposed upon other diseases such as amebiasis and schistosomiasis (Maldonado, 1946), and ulcerative colitis (Kiskaddon and Renshaw, 1945). Markell *et al.* in 1947 investigated the parasites in the stools of 1,153 military personnel and found only two cases (0.17%) of *Isospora hominis*. Magath in 1935 reported one case among 60,000 stool specimens at the Mayo Clinic; his review of 208 authenticated cases revealed that about three-fourths of them were in soldiers from the Mediterranean War Zone. This infection, however, is relatively rare in the temperate zone. Routh *et al.* in 1955 collected from the literature and summarized 27 cases from the United States. The majority of these individuals seem to have acquired the infection in foreign countries. Only one previous case has been listed from Tennessee. This was reported in a state-wide survey of parasites by Meleney, Bishop and Leathers in 1932.

The symptomatology has been constant in the various cases described. That the symptom complex was due to *Isospora* was confirmed by Matsubayashi and Nozawa (1948) by the experimental infection of a human volunteer. Another case developing from an accidental laboratory infection was described by Connal in 1922. A clear description of *Isospora hominis* is given by Humphrey (1946). The oocysts found in the stools are probably ingested with food or drink contaminated by fecal material containing fully developed oocysts.

CLINICAL MATERIAL

Case 1. D.B., March 25, 1944. The patient, a 31-year-old white married farm housewife, complained of nausea, lassitude and abdominal discomfort upon arising. On March 29, 1944, four days later, she vomited green liquid, felt chilly, and ran a fever of one degree. Three days later, the first of a series of eight to twelve foul-smelling stools began, accompanied by several chills, abdominal cramping, and bloating. Paregoric, kaolin, mild sedation, and dietary measures gave no relief. On the eighth day of the illness (April 2, 1944), a stool examination revealed a greenish-yellow foamy stool, with much mucus but no gross blood, in which *Isospora hominis* oocysts were identified. No other parasites, eggs, or cysts were found. The benzidine and guiaiac tests for occult blood were negative. On the fifteenth day (April 9, 1944), *Isospora hominis* cysts were again found. The diarrhea and other symptoms persisted until the twenty-first day of the illness. Stool examinations on the twenty-second (April 16, 1944), and thirty-sixth day (April 30, 1944) were negative. There was complete recovery without any specific therapy. A final search for the parasites on June 7, 1944, twelve weeks after the beginning of the illness, was unsuccessful.

Case 2. J.B., June 5, 1944. This patient, a ten-year-old white female student, daughter of the previous patient (Case 1), was well until twelve weeks after the beginning of the illness of the mother, when she developed severe cramps, and a watery, greenish diarrhea; fever of 103°F.; several chills; nausea, vomiting, abdominal discomfort and prostration. Dehydration ensued and on the second day required parenteral fluids. A stool examination on the third day of the illness (June 7, 1944) was negative. On the sixth day (June 11, 1944), *Isospora hominis* oocysts were found. By the end of ten days, the patient had become asymptomatic. Thirteen days after the onset (June 18, 1944), no further parasites could be demonstrated in the stool.

Case 3. M.S., February 12, 1955. This seventy-seven-year-old white married female was well until twelve hours before admission to the hospital when she experienced the sudden onset of severe abdominal pain, nausea, vomiting of greenish-yellow material ten or twelve times, and several chills. About four hours before admission she developed diarrhea which was severe and consisted of loose, watery stools with much mucus and a small amount of bright blood. Her past history was negative except for intolerance of fatty foods, with epigastric discomfort.

Physical examination upon admission showed a blood pressure of 140/90, a temperature of 102°F., pulse 110, respirations 30. In appearance the patient was well-developed and well-nourished; she was lethargic, but responded to questioning. There was marked dehydration of the skin and tongue. The abdomen was distended and tympanitic, with generalized tenderness. Peristalsis was infrequent but high-pitched.

The laboratory examinations revealed 15 grams of hemoglobin and a white blood count of 3,800. The eosinophiles were 12% on the first day, and 16% on the second. Electrolyte studies revealed a marked imbalance. Blood culture was negative. Stool examination revealed *I. hominis* oocysts and many *Trichomonas intestinalis*; many Charcot-Leyden crystals; and some occult blood. The stool culture showed no enteric pathogens. Measurement of 12 oocysts gave an average length of 23.11 microns by 13.17 microns in width. The routine agglutinations were negative, as was urinalysis.

Treatment consisted of intravenous fluids, plasma, electrolytes, paregoric, cremosuxidine, achromycin, and levarterenol added to intravenous fluids in order to maintain blood pressure. Chloromycetin, streptomycin, and Wangenstein suction was started. During the period in hospital the patient's temperature rose to 105°F., and the diarrhea persisted unabated. She remained comatose and had several episodes of generalized tonic convulsions during her last 24 hours. Patient's course was rapidly downhill and she expired on the second day in hospital.

Post-mortem examination revealed 400 cc. of clear fluid in the left pleural cavity, 300 cc. in the right, and 1,000 cc. of clear fluid containing several large flakes of light green viscid material in the abdominal cavity. Cultures and examination of the pleural and ascitic effusions were negative. The heart and lungs appeared essentially normal.

There were no areas of perforation, erosion, or palpable masses in the abdo-

men. The liver was normal. The gall bladder was intact and contained 12 stones. There was an acute necrotizing gastroenteritis with necrosis of the terminal ileum and colon. The mucosa was infiltrated with numerous acute inflammatory cells and the veins of the submucosa were dilated and contained recent thrombi. No thrombi were visible in the mesenteric veins. Examination of the remaining organs showed no remarkable changes.

DISCUSSION

Though more than 300 cases of human isosporiasis are found in the world medical literature, only 27 cases have been reported from the United States, the majority of which were probably contracted abroad. The three cases reported in this paper were native Tennesseans who had never been outside the continental United States. Two (Cases 1 and 2) had never left the boundaries of their native state.

Ordinarily, human isosporiasis is considered to be a self limited disease, subject to spontaneous recovery. This was true in Cases 1 and 2. However, Case 3, infected with *Isospora hominis*, died probably as a result of electrolyte imbalance. Postmortem examination revealed necrotizing gastro-enteritis with a necrosis of the lining of the terminal ileum and colon. There was also evidence of a sterile peritonitis without perforation of a viscus. Culture of the stool was negative for enteric pathogens. No *Isospora* or other organisms were obtained by culture of the ascitic and pleural effusions, and careful search of sections of the intestines and liver failed to disclose the presence of sporozoa in the tissue.

The report of these three cases, with the necropsy findings of one, was deemed worthwhile because of the sparsity of case reports in this country and also since no other autopsy seems to have been recorded. It has been suspected that the life cycle of the organism in man might be analogous to that in dogs and cats and that the *Isospora* might possibly be found in the mucosa of the villi of the small intestine (Smyly and Knei, 1936).

SUMMARY

Three cases of isosporiasis in native-born Tennesseans who have never been abroad are reported. Two of the cases recovered spontaneously; the third, a seventy-seven-year-old white woman, expired with signs of electrolyte imbalance. Necropsy findings showed necrosis of the terminal ileum and colon and a sterile peritonitis. The organisms could not be demonstrated in the tissues.

REFERENCES

CONNAL, A., 1922. Observations on the pathogenicity of *I. hominis*, *Trans. Roy. Soc. Trop. Med. & Hyg.* **16:** 223–245.

GERUNDO, MICHELE, 1948. Control of hepatic coccidiosis of rabbits with succinylsulfathiazole, U.S.P., *Arch. Pathl.* **46:** 128–131.

HUMPHREY, ARTHUR A., 1946. *Isospora hominis* infection in man, *J. A. M. A.* **130:** 143–145.

KISKADDON, R. M., AND RENSHAW, R. J. F., 1945. Human coccidiosis, *J. A. M. A.* **128:** 731–732.

MAGATH, THOMAS BYRD, 1935. The coccidia of man, *Am. J. Trop. Med.* **15:** 91–129.

MALDONADO, JOSÉ F., 1946. *Isospora hominis* Fantham, 1917, in Puerto Rico, *Puerto Rico J. Pub. Health & Trop. Med.* **21:** 397–400.

MARKEL 1, EDWARD K., 1950. Infection with *Isospora hominis*: report of two cases, *J. Parasitol.* **36:** 500–501.

MARKEL 2, EDWARD K., MULLINGER, PAUL E., AND SCHNEIDER, DONALD J., 1947. Intestinal infections in naval personnel, *Am. J. Trop. Med.* **27:** 63–65.

MATSUBAYASHI, HISAKITI, AND NOZAWA, TAKASHI, 1948. Experimental infection of *Isospora hominis* in man, *Am. J. Trop. Med.* **28:** 633–637.

MELENEY, H. E., BISHOP, E. L., AND LEATHERS, W. S., 1932. Investigation of *Endamoeba histolytica* and other intestinal parasites in Tennessee. 3. A state-wide survey of the intestinal protozoa of man, *Am. J. Hyg.* **16:** 523–539.

ROUTH, CONRAD, F., CROAN, JOHN E., JR., AND HAMES, CURTIS G., 1955. Three cases of human infection with *Isospora* in Georgia, *Am. J. Trop. Med. & Hyg.* **4:** 1–8.

SMYLY, H. JOCELYN, AND KNEI, CHANG, 1936. Two cases of coccidiosis in man, *Chinese M. J.* **50:** 1195–1202.

WELLS, WILLIAM H., AND BUCKI, AUGUST J., 1953. *Isospora* infection in man, *U. S. Armed Forces Med. J.* **4:** 769–772.

THE USE OF ERYTHROCYTE SENSITIZING SUBSTANCE IN THE DIAGNOSIS OF LEPTOSPIROSES

I. The Sensitized Erythrocyte Agglutination Test[1]

DOROTHY E. McCOMB, D. J. W. SMITH, DAVID L. COFFIN, ROBERT A. MacCREADY and R. SHIHMAN CHANG

Department of Microbiology Harvard School of Public Health, Boston, Massachusetts, The Laboratory of Microbiology and Pathology, Department of Health and Home Affairs Queensland, Australia and The Angell Memorial Hospital, and the Diagnostic Laboratory, Massachusetts Department of Public Health

In a previous publication from this laboratory (Chang and McComb, 1954) serologically active erythrocyte sensitizing substances, ESS, extracted from 5 strains of leptospirae were described. The ESS were capable of sensitizing erythrocytes thereby rendering the erythrocytes agglutinable by specific leptospiral antiserum. The preparations of ESS were sero-group specific and stable for 2–16 weeks, but not longer. The potential usefulness of the ESS in the diagnosis of leptospiroses was demonstrated by the positive results obtained with human leptospiroses sera. Because of the simplicity and the broad reactivity against several serotypes of leptospirae tested, the ESS appeared promising as a substitute for the laborious agglutination-lysis test (Schüffner and Mochtar, 1927). Further study was, therefore, continued to extract ESS from other leptospiral serotypes, to stabilize the ESS preparation and to evaluate the usefulness of the ESS test in the diagnosis of human leptospiroses. This publication presents the results of such a study.

The evaluation of the hemolytic modification of the leptospiral-ESS test (Cox, 1955) in the diagnosis of human leptospiroses is presented in a subsequent paper (Chang *et al.*, 1957). For clarity of presentation in this series of publications, the ESS test is referred to as the sensitized erythrocyte agglutination (SEA) test (see Megaw, 1953), and the hemolytic modification as the sensitized erythrocyte lysis (SEL) test.

MATERIALS AND METHOD

Leptospirae. All strains of leptospirae were obtained through the courtesy of Miss Julia M. Coffey, Division of Laboratories and Research, New York State Department of Health. The serotypes included in this study were *Leptospira icterohemorrhagiae, L. canicola, L. pomona, L. autumnalis, L. sejroe, L. hebdomadis L. grippotyphosa, L. pyrogenes, L. bataviae* and *L. ballum*. The leptospirae were grown by inoculating 1 ml. of a mature culture, usually 7 to 14 days old, into 20 ml. of Stuart medium containing 10% inactivated rabbit serum (Stuart, 1946). The media were incubated at 30°C. for about two weeks. The leptospirae were

[1] This work was supported in part by a research grant from the U. S. P. H. S. National Institutes of Health, Division of Research Grants, E553(C), and by a research grant from the Research Laboratories of Chas. Pfizer Co.

harvested from the culture by centrifugation at 10,000 rpm. for 30 minutes at 20 to 25°C. in a Sorvall angle head. The sedimented leptospirae were resuspended in sufficient 0.85% NaCl solution to give an optical density of 0.25 at a wave length of 650 millimicrons in the 18 mm. cuvette of a Junior Coleman spectrophotometer. This procedure standardized the number of leptospirae per ml. of diluent. The leptospirae were concentrated about 10 to 40 fold. Occasionally certain batches of leptospirae failed to resuspend evenly after the high speed centrifugation; such batches were not included in comparative studies.

Sera. Rabbit anti-leptospiral sera were prepared by immunizing rabbits with live leptospira cultures (Ezell *et al.*, 1952). Human leptospiroses sera were kindly supplied by Dr. Martha K. Ward of the Communicable Disease Center and Dr. J. I. Tonge of the Laboratory of Microbiology and Pathology, Department of Health, Queensland, Australia. Sera from patients suspected of leptospirosis were obtained from various hospitals in Boston and from Yugoslavia through the help of Dr. Edward S. Murray of this department. Routine survey sera were obtained from the Massachusetts Department of Public Health. Canine sera were obtained from the Angell Memorial Hospital in Boston. All sera were inactivated at 56°C. for 30 minutes. Rabbit and canine sera were absorbed with $\frac{1}{10}$ volume of washed packed human group "O" erythrocytes.

Erythrocytes. Human group O erythrocytes from 3 donors were used exclusively in this study. The erythrocytes were stored in Alsever's solution at 0° to 4°C., and were used within 2 weeks. On the day of a test, the erythrocytes were washed 3 times in isotonic phosphate saline solution (NaCl, 6.8 g.; Na_2HPO_4, 1.05 g.; KH_2PO_4, 0.81 g.; distilled water, 1,000 ml.; pH, 6.8).

Preparation of leptospiral-ESS. Freshly harvested and standardized leptospiral suspensions were used for the extraction of ESS. Lyophilized leptospirae or leptospiral suspensions stored without preservatives for several weeks gave irregular results. Sodium taurocholate, 0.5 gm., was dissolved in 10 ml. of leptospiral suspension. After 2 hours at 37°C., the mixture was cooled to 0–4°C. and 20 ml. of absolute ethanol, also cooled to 0–4°C., was added. A moderate amount of flocculate was formed after 2 hours at 0–4°C. This flocculate did not contain any ESS activity and was removed by centrifugation at 1,500 rpm for 10 minutes and discarded. To the supernatant, 70 ml. more of absolute alcohol was added and the mixture was kept at 0–4°C. for at least 24 hours for complete flocculation. The small amount of fine floccule was collected by centrifugation at 1,500 rpm for 10 minutes and redissolved in 10 ml. of isotonic neutral phosphate saline solution containing 1.3% formalin.

Titration of the ESS. Serial 2-fold dilutions of the ESS preparation were made in isotonic phosphate saline solution. To 1 ml. of the diluted ESS, 0.1 ml. of 10% human group O erythrocytes was added. The ESS-erythrocyte mixture was kept in a 37°C. water bath for 1 hour. The erythrocytes were kept dispersed in the diluted ESS by shaking the mixture every 15 minutes. The sensitized erythrocytes were washed twice with isotonic phosphate saline solution by centrifugation at 1,500 rpm for 5 minutes and resuspended in 1 ml. of the same diluent. 0.1 ml. of the sensitized erythrocytes were added to 0.4 ml. of a known positive serum,

diluted to 4 times its ESS antibody titer. After shaking, the tubes were kept in a 30°C. water bath for 16 to 20 hours. Agglutination was indicated by macroscopic clumps of erythrocytes upon gentle shaking of the tubes. The highest dilution of the preparation which sensitized the erythrocytes under these experimental conditions was considered one unit of ESS. When one batch of ESS is titrated at different times by this method, the endpoint may vary as much as, but not more than, 4-fold.

The leptospiral SEA test. Sensitized erythrocytes were prepared by mixing 1 part of 10% erythrocytes with 10 parts of diluted ESS containing at least 4 units of ESS per ml. The mixture was incubated and the erythrocytes were washed and resuspended as described in the previous paragraph. Serial 2-fold dilutions were made of the test sera. To 0.4 ml. of diluted serum, 0.1 ml. of 1% sensitized erythrocytes was added. The tubes were shaken and kept in a 30°C. water bath for 16 to 20 hours. Agglutination was indicated by clumps of erythrocytes upon gentle shaking of the tubes. By this method, the antibody titers of several known positive sera were reproducible within a 4-fold difference. The presence of antibodies against human erythrocytes not treated with ESS must be detected by testing the serum with 1% washed erythrocytes; when present, such antibodies were removed by absorption prior to the SEA test.

Agglutination-lysis test. The procedure of Schüffner and Mochtar (1927) was used. The lowest serum dilution used in this test was 1 to 100.

RESULTS

ESS from 9 serotypes. The extraction of sero-group specific ESS from *L. icterohemorrhagiae*, *L. canicola*, *L. pomona*, *L. hebdomadis* and *L. autumnalis* had been described (Chang and McComb, 1954). ESS with similar activity were also extracted from *L. bataviae*, *L. pyrogenes*, *L. sejroe* and *L. ballum*. We failed in three attempts to extract ESS from *L. grippotyphosa*. However, sera from rabbits immunized with *L. grippotyphosa* agglutinated erythrocytes sensitized with ESS from the other nine strains of leptospirae.

Study on methods of preserving ESS. In our previous publication (Chang and McComb, 1954), the rapid deterioration of several preparations of ESS was emphasized. Most preparations lost their activity after 2 weeks to 4 months at 0 to 4°C. Preliminary study on the preservation of ESS, involving the use of several preservatives, temperature of storage, and lyophilization, indicated that ESS preserved with 1.3% formalin stored at 0 to 4°C. gave the most favorable results. More extensive study was then conducted on the use of formalin as a preservative. In a typical experiment, ESS was extracted from a standardized suspension of leptospirae by the method described in this publication. The preparations of ESS were then stored (a) without preservatives, (b) with 1.3% formalin and (c) with 0.02% merthiolate. They were stored at 0 to 4°C. and titrated for ESS activity at regular intervals. Results are shown in Table 1, indicating that ESS preserved with 1.3% formalin contained the most activity after 41 weeks at 0 to 4°C. This experiment was repeated twice with similar results. At 37°C., a preparation of ESS preserved with 1.3% formalin showed a 4-fold decrease in activity after 28 days.

TABLE 1

Activity of ESS preparations preserved with formalin merthiolate and without preservative at 0 to 4°C.

Type of preparation	ESS activity after weeks of storage					
	0	4	9	13	18	41
ESS with 1.3% formalin	640*	160	320	160	320	160
ESS with 0.02% merthiolate	640	160	80	160	320	40
ESS without preservative	640	160	160	80	20	0

* Highest dilution of the ESS preparation capable of sensitizing a standard volume of erythrocytes.

TABLE 2

*Antibody titers of human Leptospiral sera from the Communicable Disease Center**

Name	Specimen	SEA test	Aggl.-lysis test
MIL.	1	320	8000
	2	160	2048
LAD.	1	1280 NEP†	1024
	2	1280 NEP	8000
CAD.	1	0	2048
	2	10	8000
BAD.	1	320	512
	2	320	8000
RPA	1	320	4000
	2	160	4000
LPA	1	160	128
	2	320	512
PEL.	1	640	1024
	2	640	512
DAV.	1	1280 NEP	2048
	2	1280 NEP	8000

* Sera and agglutination-lysis titers were kindly furnished by Dr. Martha K. Ward. The sera were collected from patients involved in a small epidemic of *L. canicola* infection in July and August, 1952. Time intervals between first and second specimens varied from 7 to 12 days. The sera were stored at −20°C. and ESS tests were performed in April, 1954. Agglutination-lysis titers against *L. canicola* were kindly furnished by Dr. Ward. The titer is expressed as the highest dilution of serum giving a positive test.

† NEP = Endpoint not reached.

Serum study. Paired sera from 8 patients with *L. canicola* infection were kindly furnished by Dr. Martha K. Ward of the Communicable Disease Center. Table 2 shows the results of agglutination-lysis test, supplied by Dr. Ward, and leptospiral-SEA test of those sera. Seven of these showed good correlation between the two tests.

Through the courtesy of Dr. J. I. Tonge of the Laboratory of Microbiology and Pathology, Queensland, Australia, serial specimens of sera from 19 patients with positive leptospira isolation from the blood were made available to this laboratory for testing. The sera had been stored at −20°C. for 6 months to 2

TABLE 3

*Antibody titers of human Leptospirosal sera from Australia**

Patient	Day after onset	Infecting Leptospira	SEA titer	Aggl.-lysis titer
1	7	*L. ictero*	0	0
	21		160	1000
	303		0	3000
2	5	*L. canicola*	0	0
	25		160	300
3	9	*L. canicola*		0
	85		0	1000
	141		10	1000
4	4	*L. australis*	0	0
	24		40	10,000
	45		0	1000
5	1	*L. australis*	0	0
	11		0	1000
6	5	*L. australis*	40	0
	27		160	300
7	2	*L. australis*	0	0
	37		40	300
	342		10	100
8	2	*L. pyrogenes* group	0	0
	27		0	3000
	107		10	3000
9	1	*L. pyrogenes* group	0	0
	38		40	1000
	81		40	300
10	1	*L. pomona*	0	0
	22		40	100
	65		40	300
11	3	*L. pomona*	0	0
	24		160	1000
	157		40	300
	361		0	300
12	25	*L. hyos*	40	1000
	51		40	1000
	415		10	30
13	3	*L. hyos*	0	0
	18		40	1000
	57		0	1000
	312		0	0
14	4	*L. hebdomadis* group	0	0
	20		160	300
	48		10	10,000
15	2	*L. hebdomadis* group	0	0
	63		40	300
	92		160	300
	326		40	30
16	3	*L. hebdomadis* group	0	0
	24		0	100
	52		10	300
17	4	*L. hebdomadis* group	0	0
	15		0	300
	27		0	100
18	4	Undetermined†	0	0
	27		40	300
	309		10	100
19	4	Undetermined†	0	0
	31		0	300

* These sera were kindly supplied by Dr. J. I. Tonge of the Laboratory of Microbiology and Pathology, Queensland, Australia. The sera had been stored at −20°C. for 6 months to 2 years prior to shipment to this laboratory. All cases were confirmed by isolation of leptospira from the blood of the patients. Agglutination-lysis titer against the infecting serotype of leptospirae were kindly furnished by Dr. Tonge.

† The designation of this strain has not been determined; preliminary investigations suggest that it may be a new world entity.

years prior to shipment from Australia to this laboratory. Results are tabulated in Table 3.

Three of the 19 cases had negative leptospiral SEA tests, 10 cases had low titers of not more than 1 to 40 and only 6 cases had rising titers of 1 to 160. If a four-fold rise in antibody titer be accepted as diagnostic, the leptospiral-SEA test failed in 8 out of 19 cases. Such results differed markedly from our previous work (Chang and McComb, 1954) in which the SEA test failed in 1 of the 15 leptospiroses cases. This led us to investigate the possibility of instability of of antibody against ESS. The results of that study are reported in another section of this paper.

Another evaluation study was carried out by testing paired or serial sera from patients suspected clinically of leptospirosis. Both the agglutination-lysis and the leptospiral-SEA tests were used. In the agglutination-lysis test, only *L. icterohemorrhagiae*, *L. canicola* and *L. pomona* were used. *L. sejroe* was used in addition when sera from Yugoslavia were tested. All sera were tested within a month after collection except for a few sera from Yugoslavia when a lapse of 3 to 6 months between collection and testing usually occurred. Sera from 70 patients were included in this study. Sixty-two of them were negative by both tests. Sera from 8 patients were positive by one or both tests as shown in Table 4.

In 5 of the 8 patients, there was good correlation between the tests. Patients A and B showed low ($\frac{1}{40}$) stationary titers against ESS and negative agglutination-lysis titers. Patient E showed a decreasing titer (from $\frac{1}{80}$ to $\frac{1}{20}$) against ESS and negative agglutination-lysis titer.

TABLE 4

*SEA and agglutination-lysis titers of sera from patients suspected of Leptospiroses**

Patient	Day after onset	SEA titer	Aggl.-lysis titer
A	3	40	0
	16	40	0
B	1st specimen	40	0
	2nd specimen	40	0
C	7	20	50
	11	640	3200
	18	640	800
	55	80	200
D	5	160	3200
	13	160	3200
E	1st specimen	80	0
	2nd specimen	20	0
F	1st specimen	0	not tested
	2nd specimen	320	1250
G	1st specimen	0	1250
	2nd specimen	160	6250
H	5	10	200
	12	640	12,800
	19	160	12,800

* Sera from 62 patients were negative by both tests and were not included in this table.

To determine the usefulness of the leptospiral-ESS test in survey study, sera from 98 healthy veterinarians in Massachusetts were tested by the leptospiral-ESS and the agglutination-lysis tests using *L. icterohemorrhagiae*, *L. canicola* and *L. pomona*. Two such sera had leptospiral-SEA titers of 1/40 and 1/80 but were negative in the agglutination-lysis test. Three sera had agglutination-lysis titers of 1/3200, 1/200 and 1/200 but were negative in the SEA test. Four hundred and nineteen specimens of routine prenatal blood submitted to the Massachusetts Department of Public Health were tested by the SEA test. Thirty-two were positive. Two of these gave a titer of 1/160; three, 1/80; ten, 1/40; seven, 1/20, and ten, 1/10. Of these 32 sera, only 3 were positive in the agglutination-lysis test, using *L. icterohemorrhagiae*, *L. canicola* and *L. pomona* as antigens. Two hundred and eleven serum specimens sent to the Diagnostic Laboratory of the Massachusetts Department of Public Health for *Salmonella typhosa*, brucellosis or heterophile agglutination tests were also tested by the SEA test. Twenty-six were positive with titers varying from 1/10 to 1/160; 6 of these were positive in the agglutination-lysis test using *L. icterohemorrhagiae*, *L. canicola* and *L. pomona*. Eleven of these SEA positive sera were sent to Col. Chester A. Gleiser of the Army Medical Center Graduate Service for agglutination-lysis tests against 16 leptospiral serotypes. Five were positive at low titers of questionable significance and 6 were completely negative.

Sera from 47 dogs admitted to the Angell Memorial Hospital were also tested by both the SEA and agglutination-lysis tests. Most of the dogs were suspected of leptospiroses clinically. Some were healthy, whereas others had chronic nephritis. Only one specimen from each dog was available, collected at the time of admission. Twenty-three were negative by both tests. Twenty-four were positive in one or both tests as shown in Table 5.

There was good correlation between the tests among the leptospiroses suspects. Thirteen were positive by both tests. Three were positive in the agglutination-lysis test but negative in the SEA test. One was positive in the SEA but negative in the agglutination-lysis test, and in this instance the diagnosis was confirmed at autopsy. Sera from two healthy and two nephritic dogs had positive agglutination-lysis but negative SEA tests.

Study on the instability of antibody to ESS on prolonged storage at −20°C. On account of the inferior results of the SEA test in establishing the diagnosis among the leptospiroses sera from Australia, a study was conducted to determine if antibody against leptospiral-ESS deteriorated on prolonged storage. The results showed a decrease of four-fold or more in antibody titer against ESS for most sera stored at −20°C. for several years. Of 18 sera picked at random from known positive materials sent from other laboratories, all showed a decrease in antibody against ESS of at least 4-fold after 1½ years of additional storage in this laboratory. These sera had been stored from one to several years prior to shipment to our laboratory. Eight leptospirosis sera collected locally showed no decrease after one year at −20°C. Three of 8 canine sera as well as 3 of 10 rabbit hyperimmune sera showed a 4-fold decrease after storage for 1½ years. Representative results are presented in Table 6.

TABLE 5

*SEA and agglutination-lysis antibody titers of sera from dogs with or without clinical evidence of Leptospiroses**

Dog	Clinical impression	SEA titer	Aggl.-lysis titer
D1	Leptospiroses suspect	0	31,250
D2	Leptospiroses suspect	160	1250
D3	Leptospiroses suspect	40	6250
D4	Leptospiroses suspect	640	6250
D7	Leptospiroses suspect	640	640
D8	Leptospiroses suspect	0	640
D9	Leptospiroses suspect	640	2560
D10	Leptospiroses suspect	640	0
D15	Leptospiroses suspect	80	3200
D16	Leptospiroses suspect	80	3200
D19	Leptospiroses suspect	160	800
D23	Leptospiroses suspect	160	3200
D24	Leptospiroses suspect	20	3200
D27	Leptospiroses suspect	40	200
D28	Leptospiroses suspect	160	800
D31	Leptospiroses suspect	0	800
D40	Leptospiroses suspect	160	1600
D39	chronic nephritis	0	800
D41	chronic nephritis	0	400
D12	no disease	0	200
D13	no disease	0	200

* All sera were collected from dogs admitted to Angell Memorial Hospital in Boston. Sera were tested within a month after collection. Only *L. icterohemorrhagiae* and *L. canicola* were used in the agglutination-lysis tests. Twenty-three sera were negative by both tests and were not included in this table.

Antibody absorption study. In view of the fact that agglutination-lysis antibody is generally known to be stable on prolonged storage and that antibody against leptospiral-ESS is relatively unstable, a study of antibody absorption was conducted to determine whether antibodies responsible for the 2 types of reaction are different. Human, canine and rabbit leptospirosis sera were diluted 1 to 10 and divided into 3 equal portions. To the first, second and third portions, equal volumes of 0.85% NaCl solution, 5% suspension of ESS treated erythrocytes and a heavy suspension of washed *L. canicola* were added respectively. After ½ hour at 37°C. and 18 hours at 4°C. the supernates were tested for antibody by the agglutination-lysis and the leptospiral SEA tests. Results are shown in Table 7; ESS removed antibody against ESS without affecting agglutination-lysis titers while suspensions of live leptospirae removed both.

Study on human leptospirosis sera stored for 3 to 12 months at −20°C. On account of the deterioration of ESS antibody on storage at −20°C., evaluation of the SEA test was carried out with a group of human leptospiroses sera not included in Table 3 which were stored at −20°C. for 3 to 12 months. The sera were generously supplied by Dr. J. I. Tonge of the Laboratory of Microbiology and Pathology, Queensland, Australia. The sera were paired and showed rising anti-

TABLE 6

Instability of Leptospiral-SEA antibody on prolonged storage at −20°C.

Serum	Length of storage prior to 1st testing	Interval between 1st and 2nd testing	SEA antibody titer	
			1st testing	2nd testing
		yrs.		
Human #1	1.5 yrs.	1.5	320	80
Human #2	1.5 yrs.	1.5	1280	160
Human #3	1.5 yrs.	1.5	320	40
Human #4	1.5 yrs.	1.5	1280	160
Human #5	several yrs.	1.75	1280	160
Human #6	several yrs.	1.75	1280	160
Human #7	several yrs.	1.75	40	0
Human #8	1 week	1	640	640
Human #9	1 week	1	640	320
Human #10	1 week	1	160	160
Dog #1	1 week	1.5	160	10
Dog #2	1 week	1.5	640	80
Dog #3	1 week	1.5	160	320
Rabbit #1	1 week	2	160	40
Rabbit #2	1 week	2	1280	160
Rabbit #3	1 week	1.5	80	0
Rabbit #4	1 week	1.5	320	160

TABLE 7

Results of antibody absorption tests

Serum	Antigen used for absorption	Antibody titers after absorption	
		SEA	Aggl.-lysis
Human	Saline	320	800
	ESS treated erythrocytes	10	800
	leptospira suspension	0	0
Rabbit	Saline	160	12,800
	ESS treated erythrocytes	0	12,800
	leptospira suspension	0	200
Dog	Saline	40	12,800
	ESS treated erythrocytes	0	12,800
	leptospira suspension	0	3200

body titers by the agglutination-lysis tests. Forty pairs of such sera were tested by the SEA test; 29 showed a 4-fold or more rise in antibody titer, 4 showed a rise from negative to $\frac{1}{10}$ or $\frac{1}{20}$ and 7 were entirely negative. Those sera showing negative or weak positive SEA test were also low in the agglutination-lysis titer. Since this study was part of a comparison between the sensitized erythrocyte agglutination test, the sensitized erythrocyte lysis test and the agglutination-lysis test, the details are presented in the next paper of this series of publication (Chang *et al.*, 1957).

DISCUSSION

The sero-group specificity of ESS extracted from the leptospirae has been further confirmed by the extraction from *L. bataviae*, *L. sejroe*, *L. pyrogenes* and *L. ballum* of an active component with serological activity similar to ESS from *L. icterohemorrhagiae*, *L. canicola*, *L. pomona*, *L. hebdomadis* and *L. autumnalis* reported previously (Chang and McComb, 1954). It is interesting that we failed to extract ESS from *L. grippotyphosa* in 3 separate trials, although *L. grippotyphosa* antisera agglutinated erythrocytes treated with ESS extracted from other serotypes. This suggests that the method recommended for the extraction of ESS may occasionally fail to yield positive results.

The stability of ESS preparation has been significantly prolonged by the extraction of fresh leptospiral suspension, the removal of the inactive components insoluble in 50% alcohol and the preservation with 1.3% formalin. Sufficient active components for the satisfactory performance of SEA test remained in such ESS preparations for at least 41 weeks.

The data on the evaluation of the leptospiral SEA test in the diagnosis of leptospiroses indicate that the antibody against ESS deteriorates on storage at −20°C. for more than 1 to 1.5 years. The correlation between the SEA and the agglutination-lysis tests was reasonably satisfactory when fresh sera were tested, but the number of sera included in this study was small. With sera from Australia, which had been stored at −20°C. for 3 months to 2 years, 19 out of 46 cases gave only weak positive or negative SEA test.

The data indicate also that antibody against ESS disappears rather rapidly after convalescence from leptospiral infection, which means that, as a test to survey the incidence of past infection by leptospirae, the ESS test is definitely inadequate. In a survey study of sera from 98 healthy veterinarians, 3 showed significant agglutination-lysis titers but the SEA test was completely negative. Sera from 2 healthy dogs and 2 chronic nephritic dogs also agglutinated leptospirae in significant titers but failed completely to agglutinate ESS treated erythrocytes.

The interpretation of the SEA test is complicated by the fact that 32 of the 419 routine prenatal bloods and 26 of the 211 sera submitted to the Massachusetts Department of Public Health for *S. typhosa*, *Brucella* or heterophile agglutination were positive in this test at low titers. Only 9 of these 58 specimens agglutinated *L. icterohemorrhagiae*, *L. canicola* or *L. pomona*. We have noted that the percentages of SEA positive sera collected from various localities vary greatly (Chang and McComb, unpublished data): 8 per cent of 630 Massachusetts sera, 0 per cent of 90 sera from New Hampshire, 1 per cent of 91 from Portugal, 0 percent of 50 from Yugoslavia and 46 per cent of 104 from Venezuela were positive. We are unable to ascertain the significance of a weak positive (titer of $\frac{1}{40}$ or less) SEA test on a single specimen of serum.

SUMMARY

A modified method for the extraction of ESS from leptospirae has been developed which yields preparations stable for at least 41 weeks at 4°C. ESS anti-

body apparently deteriorated on storage at −20°C. for more than 1 to 1.5 years. Good correlation between the SEA and the agglutination-lysis tests were obtained with fresh sera. Unsatisfactory results were obtained with sera shipped from Australia. About 8 per cent of sera sent to the Massachusetts Department of Public Health for various diagnostic tests were positive in the SEA test, the significance of which is not ascertained. Different antibodies are responsible for the SEA and the agglutination-lysis reactions.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. John C. Snyder for his interest and suggestions; to Dr. J. I. Tonge for his interest and generous supply of leptospiroses sera; to Dr. Martha K. Ward for her generous supply of leptospiroses sera; and to Lt. Col. Chester A. Gleiser for his help in some of the agglutination-lysis tests.

REFERENCES

CHANG, R. S. AND McCOMB, D. E., 1954. Erythrocyte sensitizing substances from 5 strains of leptospirae, *Am. J. Trop. Med. and Hyg.* **3:** 481–489.

CHANG, R. S., SMITH, D. J. W., McCOMB, D. E., SHARP, C. F. AND TONGE, J. I. The use of erythrocyte sensitizing substances in the diagnosis of leptospiroses. II. The sensitized erythrocyte lysis test, *Am. J. Trop. Med. and Hyg.* (same issue)

COX, C. D., 1955. Hemolysis of sheep erythrocytes sensitized with leptospiral extracts, *Proc. Soc. Exp. Biol. and Med.* **90:** 610–615.

EZELL, S. B., HOAG, W. G., WARNER, A. R., YAGER, R. H., AND GOCHENOUR, H. S., 1952. Soluble specific leptospiral complement-fixing antigens, *Proc. Soc. Exp. Biol. and Med.* **80:** 220–223.

MEGAW, J. W. D., 1953. Abstract on "Erythrocyte-sensitizing substances from the typhus rickettsiae", *Trop. Dis. Bull.* **50:** 613–614.

SCHÜFFNER, W. AND MOCHTAR, A., 1927. Versuche zur Aufteilung von Leptospirenstammen, mit einleitenden Bemerkungen ueber den Verlauf van Agglutination und Lysis, *Cent. f. Bakt. I. Abt. Orig.* **101:** 405–413. (Abstracted in *Trop. Dis. Bull.* 1927, **24:** 714.)

STUART, R. D., 1946. The preparation and use of a simple culture medium for leptospirae, *J. Path. Bact.* **58:** 343–349.

THE USE OF ERYTHROCYTE SENSITIZING SUBSTANCE IN THE DIAGNOSIS OF LEPTOSPIROSES

II. The Sensitized Erythrocyte Lysis Test[1]

R. SHIHMAN CHANG, D. J. W. SMITH, DOROTHY E. McCOMB, C. F. SHARP AND J. I. TONGE

Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts and the Laboratory of Microbiology and Pathology, Department of Health and Home Affairs, Queensland, Australia

In the preceding article (McComb *et al.*, 1957) data on the evaluation of the leptospiral sensitized erythrocyte agglutination (SEA) test in the diagnosis of leptospiroses were presented. Unsatisfactory results were obtained with leptospiroses sera shipped to the Harvard Laboratory from Australia. Recently, Cox (1955) described a hemolytic modification of the SEA test which was apparently more sensitive in the detection of leptospiral antibody. A comparative study of the SEA test and its hemolytic modification in the diagnosis of human leptospiroses was therefore initiated in our Laboratory. Serial serum specimens collected from 40 cases of human leptospiroses with diagnosis proved by positive blood cultures for leptospirae or by the agglutination-lysis test were titrated for leptospiral antibodies by the two tests under study. In this publication such results are presented. For clarity in presentation, the leptospiral extract capable of sensitizing erythrocytes is described as leptospiral erythrocyte sensitizing substance (ESS); the agglutination of ESS-treated erythrocytes by specific antiserum is called the sensitized erythrocyte agglutination (SEA) test, (see Megaw 1953); and the lysis of ESS-treated erythrocytes in the presence of specific antiserum and complement, is called the sensitized erythrocyte lysis (SEL) test.

MATERIALS AND METHODS

The growth and standardization of leptospirae, the preparation and titration of ESS as well as the procedure for the SEA test were described in the preceding article (McComb *et al.*, 1957). Technics used in the isolation of leptospirae from the blood of patients suspected of leptospiroses, the typing of leptospirae isolated and the agglutination-lysis test have also been described (Smith *et al.*, 1954). Thirteen leptospiral serotypes, known to occur in Australia, were included as antigens in the agglutination-lysis test. They were *L. icterohemorrhagiae*, *L. canicola*, *L. pomona*, *L. hyos*, *L. australis* A, *L. australis* B, *L. grippotyphosa*, *L. medanensis*, "Robinson", "Kremastos", "Szwajizak", "Esposito", and "Celledoni". (Preliminary investigations suggest that the last 5 strains may represent new serotypes.)

The sensitized erythrocyte lysis (SEL) test. The procedures described by Fisher

[1] This work was supported in part by a research grant from the U. S. P. H. S. National Institutes of Health, Division of Research Grants, E553(C), and by a research grant from the Research Laboratories of Chas. Pfizer Co.

(1950), Middlebrook (1950), and Cox (1955) were used with minor modifications. For the convenience of the readers, the procedure is described in detail. Materials required in the SEL test were as follows:

1. *Diluent.* The Mg-barbiturate buffer described by Mayer and co-worker (1946) was used in this test as sole diluent. It consisted of 85 gm. of NaCl, 5.75 gm. of 5,5-diethyl barbituric acid, 3.75 gm. of sodium 5,5-diethyl barbiturate, 1 gm. of $MgCl_2$-$6H_2O$ and 0.2 gm. of $CaCl_2 \cdot 2H_2O$ in 10 liters of distilled water.

2. *Sheep erythrocytes.* Sheep blood was preserved aseptically in equal volumes of Alsever's solution (2 gm. of glucose, 0.8 gm. of sodium citrate and 0.42 gm. of sodium chloride in 100 ml. distilled water; pH adjusted to 6.1 with citric acid). For use in the test, the cells were washed three times in Mg-barbiturate buffer by centrifugation at 2,000 rpm for 15 minutes.

3. *Complement.* Commercially prepared lyophilized guinea pig serum was used. The desiccated complement was reconstituted to the original volume with the chilled diluent supplied by the manufacturer and frozen at −60°C. in small aliquots. If preservation of the complement at −60°C. was not feasible, storage at −20°C. was also satisfactory in preserving its activity for at least 4 months (Osler *et al.*, 1952). Unlike the complement fixation test, the exact amount of complement used in the SEL test was not important, providing an excess was used. We found that a dilution of 1/30 to 1/60 of complement represented a suitable concentration for the SEL test. At this dilution, the amount of sheep erythrocyte antibody present in most guinea pig sera was sufficiently diluted so as to make the absorption of complement by sheep erythrocytes at 0–4°C. unnecessary.

4. *Sera.* All sera were heated at 56°C. for 30 minutes, diluted 1 to 5, mixed with one-tenth volume of washed packed sheep erythrocytes at 37°C. for 15 minutes, then centrifuged at 1,500 rpm for 15 minutes to remove the sheep erythrocytes. This step was essential to remove sheep erythrocyte antibody present in most human sera.

5. *ESS-treated erythrocytes.* This procedure has already been described (McComb *et al.*, 1957). ESS prepared from *L. pomona* was used exclusively. We consistently used an excess of ESS to eliminate the step of ESS titration. One ml. of ESS diluted 1/10 adequately sensitizes 0.1 ml. of washed packed sheep erythrocytes.

The SEL test was carried out by adding 0.1 ml. of 1% sensitized erythrocyte and 0.5 ml. of complement diluted 1/30 to 0.4 ml. of serial fourfold dilution of serum. After agitation to insure thorough mixing, the tubes were incubated for one hour in a 37°C. water bath. Complete, partial, or no hemolysis was then recorded. In the presence of sufficient antibody, complete hemolysis occurred. Tubes showing partial or no hemolysis indicated the absence of antibody. Controls consisted of: (a) test serum diluted 1/10, unsensitized sheep erythrocyte and complement, (b) leptospiral antiserum diluted to 4 times its titer (for example, if the SEL titer was 1/2,560, the serum was diluted 1/640), sensitized erythrocytes and complement; and (c) known negative serum, sensitized erythrocytes

and complement. There should be no hemolysis in controls *a* and *c*, and complete hemolysis in *b*.

RESULTS

Serum study. Paired sera from 40 proven cases of human leptospiroses occurring in Australia were tested for leptospiral antibody by the SEA and SEL tests. The diagnosis of leptospiroses in 26 cases was established by the isolation of leptospirae from the blood and the agglutination-lysis test. With the remaining 14 cases, blood cultures either failed to yield any leptospirae or were not attempted and the clinical diagnoses were confirmed by the agglutination-lysis test. Using the SEA test, 29 cases showed four-fold or more rise in antibody titer. Four showed a rise from 0 to $\frac{1}{10}$ or $\frac{1}{20}$, and 7 were completely negative. On the other hand, the SEL tests were positive in all cases and the titers were higher. The serogroup reactivity of the ESS was once again demonstrated. Using ESS extracted from *L. pomona*, the SEL tests detected marked rise of antibody titers in paired sera collected from individuals infected by *L. icterohemorrhagiae*, *L. canicola*, *L. australis* A, *L. australis* B, *L. pomona*, *L. hyos*, "Robinson", "Kremastos", and "Celledoni".

To ascertain the specificity of the leptospiral SEL test, paired acute and convalescent sera collected from 5 cases of Brill-Zinsser disease, 2 cases of rickettsialpox, 1 case of Rocky Mountain spotted fever, 1 case of fièvre boutonneuse, 2 cases of murine typhus, 3 cases of scrub typhus, 2 cases of rubella, 6 cases of infectious hepatitis and 20 cases of undiagnosed fever were tested for leptospiral antibodies by the SEL test. None of these 42 pair of sera showed rising antibody titers. Twelve of them showed stationary SEL titers of $\frac{1}{10}$ to $\frac{1}{160}$. Four anti-*Treponema pallidum*, 1 anti-Reiter's spirochete and 2 anti-*Borrelia novyi* rabbit sera were also negative for leptospiral SEL antibody.

To determine the value of SEL test in survey studies on past leptospiral infection, the SEA and SEL tests were performed on 50 sera submitted to the Massachusetts Department of Public Health for Wassermann tests. Only one dilution (1/10) of sera was used. Eighteen were negative in both tests; 3 were positive in both tests; 29 were positive in the SEL but negative in the SEA test; and none were positive in the SEA and negative in the SEL test.

A comparison of the sensitivity of sheep and human group "O" erythrocytes in the SEA and SEL tests. Since sheep erythrocytes were used in the SEL test, it was essential to remove heterophile antibodies from all sera by absorption with sheep erythrocytes. In an attempt to eliminate this laborious step, a comparison of the sheep and human group "O" erythrocytes in the SEL and SEA tests was carried out. It was found that when human instead of sheep erythroytes were used, the SEL antibody titers of three positive sera were 64-fold lower and the amount of complement required was about 6 times higher. On the other hand the SEA titers were similar whether human or sheep erythrocytes were used.

Serum absorption study. In view of this difference in the SEL antibody titers obtained with human and sheep erythrocytes, antibody absorption study was

TABLE 1

Leptospiral antibody titers of human leptospiroses sera by the SEA, SEL and agglutination-lysis tests

Case	Day after onset	Serotype isolated	Antibody titers*		
			SEA	SEL	Aggl.-lysis
18	3	*L. australis* B.	0	0	0
	22		10	2,560 NEP	300
19	5	*L. australis* B.	0	0	0
	29		160	2,560 NEP	1,000
39	2	*L. australis* A.	0	0	0
	19		160	2,560 NEP	100
43	1	*L. australis* A.	0	0	0
	26		10	640	1,000
26	2	"Robinson"	0	0	0
	21		160	2,560 NEP	100
52	3	"Robinson"	0	0	0
	21		10	2,560 NEP	100
33	6	*L. hyos*	0	40	0
	17		640 NEP	2,560 NEP	3,000
38	2	"Kremastos"	0	0	0
	24		40	2,560 NEP	1,000
56	4	"Kremastos"	0	0	10
	25		40	160	300
46	3	*L. icterohemorrhagiae*	0	0	0
	14		160	2,560 NEP	3,000
37	2	*L. canicola*	0	0	0
	21		0	640	300
44	2	*L. canicola*	0	0	0
	26		40	640	100
12	8	None†	0	40	0
	21		40	2,560 NEP	1,000
13	6	None	0	0	0
	22		40	2,560 NEP	1,000
14	6	None	0	0	0
	22		40	2,560 NEP	1,000
6	6	None	0	40	0
	35		0	640	300
7	3	None	0	0	30
	14		0	640	100
8	7	None	0	10	0
	14		40	2,560	300
9	9	None	0	160	30
	17		20	2,560	1,000
10	5	None	0	40	0
	18		160	2,560 NEP	3,000
11	8	None	0	0	100
	22		0	40	1,000
1	7	None	0	10	0
	17		640	2,560 NEP	300
2	5	None	0	0	0
	21		80	640	1,000

TABLE 1—*Continued*

Case	Day after onset	Serotype isolated	Antibody titers*		
			SEA	SEL	Aggl.-lysis
3	4	None	10	0	0
	11		640	2,560 NEP	1,000
4	4	*L. australis* A.	0	0	0
	30		0	160	10,000
5	1	None	0	0	0
	78		0	160	1,000
15	7	None	0	160	100
	22		40	2,560 NEP	10,000
25	4	*L. australis* B.	0	0	10
	24		160	2,560 NEP	1,000
34	4	*L. australis* B.	0	0	0
	19		40	160	1,000
35	2	*L. australis* B.	0	0	10
	26		640	640	300
47	4	*L. australis* B.	0	0	0
	23		0	160	300
48	4	*L. australis* A.	0	0	0
	25		40	640	3,000
51	4	*L. australis* B.	0	0	0
	20		40	40	3,000
53	2	*L. australis* B.	0	160 NEP	300
	22		160	640	300
54	3	*L. australis* B.	0	10	0
	32		40	640	1,000
58	3	*L. australis* B.	0	0	0
	26		160	2,560 NEP	100
59	5	"Kremastos"	0	160	0
	160		160	2,560 NEP	1,000
60	1	*L. australis* B.	0	0	0
	15		640	2,560 NEP	300
61	2	"Kremastos"	0	0	0
	27		160	2,560 NEP	300
62	3	"Kremastos"	0	0	0
	23		40	640	1,000

* Titers were expressed as the highest dilution of sera giving a positive reaction.
† Blood cultures either failed to yield any leptospirae or were not attempted.

performed to determine if the SEA and SEL involved the same antigen-antibody reaction, and if sheep and not human erythrocytes absorbed antigens other than the ESS responsible for the SEA test. Human and rabbit leptospiroses sera were diluted 1 to 5 and distributed into 3 one ml. portions. To 1st, 2d and 3d portion, 1 ml. of diluent, 5% ESS-treated human and sheep erythrocytes were added respectively. After 1 hour at 37°C., the sera were tested for antibody by the SEA and SEL tests using ESS-treated human and sheep erythrocytes respectively. Results showed that SEA and SEL antibodies of leptospiroses sera were removed by absorption with ESS-treated erythrocytes, human or sheep.

TABLE 2

The SEL and SEA titer of sera tested with ESS-treated sheep and human erythrocytes

Sera	Type of test	Antibody titer	
		Sheep cell*	Human cell*
Rabbit anticanicola	SEA	40	40
	SEL	10,240	40
Human leptospiroses 1	SEA	160	160
	SEL	2,560	40
Human leptospiroses 2	SEA	160	160
	SEL	2,560	40
Normal rabbit	SEA	0	0
	SEL	0	0

* Complement diluted 1/60 and 1/10 was used for sheep and human erythrocytes respectively.

TABLE 3

Antibody absorption study

Antiserum	Antigen used in absorption	SEA[1] titer	SEL[2] titer
Rabbit serum	Diluent	40	10,240
	ESS-treated human cells	<10	<10
	ESS-treated sheep cells	<10	<10
Human serum	Diluent	320	20,480
	ESS-treated human cells	<10	80
	ESS-treated sheep cells	<10	<20

[1] Human erythrocytes were used.
[2] Sheep erythrocytes were used.

DISCUSSION

The results of this study clearly establish the diagnostic value of the SEL test. The test is sensitive in establishing the diagnosis of leptospiroses and in obtaining high antibody titers. Using rising antibody titers of paired acute and convalescent sera as the diagnostic criteria, the SEL test established the diagnosis in all 40 cases of proven human leptospiroses and was negative in 42 cases of various non-leptospiral infection. Titers in the positive cases were high, varying from $\frac{1}{160}$ to over $\frac{1}{2560}$. The antibody rise in acute and convalescent sera was also very marked, 16 to more than 256 fold. Its broad reactivity against all leptospiral sero-types studied was demonstrated by Cox (1955) with rabbit sera. This study reconfirmed its sero-group specificity in natural human infection.

The significance of a positive SEL test on a single specimen of serum is not clear. Of the 42 cases of non-leptospiral infection 12 (30%) gave stationary SEL titers varying from $\frac{1}{10}$ to $\frac{1}{160}$. Of 50 sera submitted to the Massachusetts Department of Public Health for Wassermann test, 32 (64%) were positive. We are unable to explain such findings. More study is required to determine whether the positive SEL reaction always indicates past contact with the leptospira,

whether contact with the non-pathogenic leptospira may also give rise to a positive SEL reaction, and whether other factors unrelated to the leptospira may be responsible for a large percentage of positive SEL test.

The correlation between the SEL and SEA titers strongly indicates that the same basic mechanism of ESS-antibody reactions are involved and that the SEL test is more sensitive, requiring fewer ESS-antibody molecular reactions to give a positive reading. The results of serum absorption study substantiate this hypothesis.

The greater sensitivity of sheep over human erythrocytes in the SEL test but not in the SEA test deserves further exploration.

SUMMARY

The leptospiral sensitized erythrocyte lysis test established the diagnosis in all 40 cases of human leptospiroses. The sero-group specificity of the test was substantiated by the ability of *L. pomona* ESS to detect human infection caused by *L. icterohemorrhagiae*, *L. canicola*, *L. pomona*, *L. hyos*, *L. australis* A, *L. australis* B, "Robinson", "Kremastos", and "Celledoni". The value of the SEL test in survey studies of past leptospiral infection requires further study.

ACKNOWLEDGEMENT

The authors are grateful to Dr. John C. Snyder for his interest and suggestions in this work.

REFERENCES

Cox, C. D., 1955. Hemolysis of sheep erythrocytes sensitized with leptospiral extracts, *Proc. Soc. Exp. Biol. and Med.* **90:** 610.

Fisher, S., and Keogh, E. V., 1950. Lysis by complement of erythrocytes which have absorbed a bacterial component and its antibody, *Nature* **165:** 248.

Mayer, M. M., Osler, A. G., Bier, O. G., and Heidelberger, M., 1946. The activating effect of magnesium and other cations on the hemolytic function of complement, *J. Exp. Med.* **84:** 535.

McComb, D. E., Smith, D. J. W., Coffin, D. L., McCready, R. A., and Chang, R. S., 1956. The use of erythrocyte sensitizing substance in the diagnosis of leptospiroses. I. The sensitized erythrocyte agglutination test, *Am. J. Trop. Med. & Hyg.* **6:** 90.

Megaw, J. W. D., 1953. Abstract on "Erythrocyte-sensitizing substances from the typhus rickettsiae", *Trop. Dis. Bull.* **50:** 613.

Middlebrook, B., 1950. A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigens, *J. Clin. Investigation* **29:** 1480.

Osler, A. G., Strauss, J. H., and Mayer, M. M., 1952. Diagnostic complement fixation, *Am. J. Syphilis* **36:** 140.

Smith, D. J. W., Brown, H. E., Tonge, J. I., Sinnamon, C. N., MacDonald, V. M., Ross C. J., and Doherty, R. L., 1954. The serological classification of 89 strains of leptospirae from North Queensland, including five serotypes new to Australia, *Aust. Ann. Med.* **3:** 98.

THE PORTAL PRESSURE IN HEPATIC FIBROSIS ASSOCIATED WITH BILHARZIASIS

M. A. ARAFA, ERNEST BIBAWI AND A. RAAFAT

Kasr El Aini Faculty of Medicine, Cairo University, Cairo, Egypt

Several methods of estimating the portal pressure have been described, e.g., catheterization of a hepatic vein (Paton *et al.*, 1953), puncture of esophageal varices during esophagoscopy (Allison, 1951) directly by puncture of venous radicle at operation, by percutaneous intrasplenic method (Atkinson and Sherlock, 1954) and by percutaneous transhepatic method (Lemaire and Housset, 1955).

In this work the validity of the intrasplenic pressure measured by the percutaneous method as an index of portal pressure was confirmed and an attempt has been made to find out the relation between the height of the intrasplenic pressure and the size of the liver and spleen, the occurrence of hematemesis, esophageal varices, venous hum, ascites and changes in the portal bed demonstrated by spleno-portal venography (Dreyer and Budtz-Olsen, 1952).

CLINICAL MATERIAL AND METHODS

Seventy-four patients with hepatic fibrosis associated with *Schistosoma mansoni* infection were examined; 28 had ascites. The diagnosis of the hepatic pathology was confirmed by examination of liver material obtained by biopsy needle or during operation. The occurrence of hematemesis, ascites, venous hum over the abdomen and lower chest and esophageal varices (roentgenologically demonstrated after a barium swallow) and the size of the liver and spleen were recorded. All patients had a normal prothrombin time, blood platelet count and bleeding and coagulation times. The percutaneous intrasplenic pressure was measured in all cases, direct intrasplenic and portal pressure measurements during operation were also made in 8 and the percutaneous transhepatic portal pressure in five. Percutaneous spleno-portal venography (Dreyer and Budtz-Olsen, 1952) was done in 27 cases.

These investigations were also carried out on 5 normal control cases and on 5 patients with splenomegaly due to other causes than hepatic fibrosis or cirrhosis, viz., Still's disease, chronic myeloid leukemia, subacute bacterial endocarditis (one case of each) and Hodgkin's disease (two cases).

RESULTS

The intrasplenic pressure in the control group of cases varied between 50 and 160 mm. saline. There was no significant difference in pressure between the normal control cases and the control cases with splenomegaly due to other causes than hepatic fibrosis or cirrhosis.

In patients with hepatic fibrosis, the intrasplenic pressure was normal (up to 160 mm. saline) in 20 (27.2%), slightly raised (160–200 mm. saline) in 7 (9.4%), moderately raised (200–250 mm. saline) in 9 (12.1%), and high (above 250 mm.

TABLE 1

Findings in patients with hepatic fibrosis

Pressure (mm. saline)	No. of cases	No. of cases showing			
		Ascites	Hematemesis	Esophageal varicosities	Venous hum
Up to 160	20 (27.2%)	2	—	—	—
160–200	7 (9.4%)	1	—	—	—
200–250	9 (12.1%)	3	—	—	—
Above 250	38 (51.3%)	22	9 (23.7%)	16 (42.1%)	3 (8%)
Total	74	28 (38)%	9(12.2%)	16 (21.6%)	3 (4%)

TABLE 2

Relation between intrasplenic and portal pressures (mm. saline)

Case No.	Percutaneous intrasplenic pressure	Direct intrasplenic pressure at operation	Portal pressure at operation
1	172	170	160
2	150	146	130
3	145	140	134
4	340	300	292
5	176	160	150
6	300	283	276
7	220	220	212
8	370	360	350

saline) in 38 (51.3%). Raised intrasplenic pressure occurred in 60 per cent of cases without ascites and in 93 per cent of cases with ascites. A summary of the findings is given in Table 1. The intrasplenic pressure measured by the percutaneous method agreed closely with that measured during operation; similarly there was a direct relation between it and the portal pressure measured directly during operation (Table 2), the latter being 8 to 48 mm. saline less than the former. A similar relation was present between the intrasplenic pressure and the portal pressure measured by the percutaneous transhepatic method.

The intrasplenic pressure was steady without pulsations, and variations with quiet respiration did not exceed 5 mm. saline. The pressure varied little from place to place in the same spleen. In two cases the needle entered an artery and a marked rise of pressure with prominent pulsations was obtained. This error was prevented by changing the position of the needle in every case and taking two pressure readings.

Hematemesis occurred in 9 (12.2%), esophageal varices in 16 (21.6%) and venous hum over the abdomen in 3 patients; all of these had high intrasplenic pressure (above 250 mm. saline). Ascites was frequently, but not invariably, associated with high intrasplenic pressure; of the 28 patients with ascites, 2 had normal, 1 slightly raised, 3 moderately raised and 22 high pressures. The size of the liver and spleen had no constant relation to the height of the intrasplenic pressure, although shrunken livers and huge spleens were usually met with when

FIG. 1. Normal spleno-portal venogram showing normal sized splenic and portal veins and the intrahepatic ramifications which can be traced up to the fifth order of division.

FIG. 2. Spleno-portal venogram in a patient with moderate rise of the intrasplenic pressure (220 mm. saline).

the pressure was moderately or greatly raised. There was no evidence that there was any relationship between the histological findings in the liver biopsy and the height of the portal pressure.

Percutaneous spleno-portal venography was done in 27 cases. In normal livers

FIG. 3. Spleno-portal venogram in a patient with high intrasplenic pressure (360 mm. saline) showing dilatation and tortuosity of the splenic and portal veins and non visualization of the intrahepatic ramifications.

with normal intrasplenic pressure the intrahepatic ramifications of the portal vein can be traced up to the 4th or 5th order of division (Fig. 1). In portal hypertension, the splenic and portal veins are dilated and tortuous, the intrahepatic ramifications could be traced to the 2nd or 3rd order of division in cases with slightly or moderately raised pressures (Fig. 2) and were not visualised at all in cases with high pressures (Fig. 3). In some cases filling of the tributaries of the portal veins, particularly those leading to the collateral circulation, e.g. the left gastric and inferior mesenteric veins, occurred. Splenic vein thrombosis occurred in 4 patients, all of whom had pressures above 300 mm. saline.

No complications of splenic puncture occurred except in one patient who developed intraperitoneal bleeding but rapidly responded to conservative treatment.

DISCUSSION

Intrasplenic pressure bears a close relation to the portal venous pressure measured by other methods. Atkinson and Sherlock (1954) believe that by measuring the intrasplenic pressure it is possible to predict the portal venous pressure within 8 mm. Hg with 95 per cent degree of accuracy and that it exceeds the latter by about 2 to 6 mm.Hg; the higher the pressure the greater the difference. This is not surprising as the needle introduced into the spleen probably lies in the splenic pulp which communicates freely with the portal system. The technique has many practical applications, viz., in diagnosis of portal hypertension, splenomegaly and gastro-intestinal bleeding of unknown nature and when used in conjunction with

catheterization of a hepatic vein or the transhepatic method of measuring the portal pressure. It may be useful for diagnosis of obstruction of the portal vein, as the intrasplenic pressure will be much higher than the portal pressure. Repeated post-operatively after portacaval anastomosis it is an indication of the efficacy of the operation and patency of the shunt. Spleno-portal venography can be done through the same needle used for measuring the intrasplenic pressure. It is valuable in diagnosis of portal hypertension and in demonstration of veins suitable for portal systemic anastomosis, as thrombosis of the portal vein is said to occur in at least 11 per cent of all cirrhotic patients (Hunt and Whittard, 1954). It can also be used to demonstrate patency of the portacaval shunt.

Splenic puncture is a relatively safe procedure and complications are uncommon and include intraperitoneal hemorrhage, injury to intra-abdominal organs, and infection. It is contraindicated in the presence of prolonged bleeding, coagulation and prothrombin times; splenic infection; recent splenic infarction (as the spleen is friable); splenic tenderness (as the capsule is tense) and in the presence of skin infection at the site of puncture.

Hematemesis occurred in about 12 per cent of cases. Bibawi *et al.* (1955) reported it in one series in 14 per cent and in another large series in 17 per cent of patients with hepatic fibrosis associated with *Schitosoma mansoni* infection. Hematemesis, esophageal varicosities and venous hum over the abdomen and lower chest were always associated with high intrasplenic pressure, but neither the size of the liver and spleen nor the occurrence of ascites is an indication of the level of the portal pressure. This agrees with the experience of Walker (1952) who found that the clinical diagnosis of portal hypertension usually rests on the demonstration of esophageal varicosities and that in intrahepatic obstruction by fibrosis the average portal pressure was higher in patients who bled from the esophagus than in those who had ascites.

SUMMARY

Seventy-four patients with heptaic fibrosis associated with *Schistosoma mansoni* infection were examined. The intrasplenic pressure measured by the percutaneous method had a direct linear relation with the portal pressure measured directly at operation and by the percutaneous transhepatic method. Hematemesis, esophageal varicosities and venous hum over the abdomen are indications of the level of the portal pressure. The size of the liver and spleen had no constant relation to the portal pressure. Ascites is frequently, but not always, associated with high pressures. Spleno-portal venography used in conjunction with intrasplenic pressure measurement is particularly valuable in diagnosis of portal hypertension and in demonstration of veins suitable for portocaval anatomosis.

REFERENCES

Allison, P., 1951. Measurements of blood pressure in oesophageal varices, *Thorax* **6**: 325–327.

Atkinson, M., and Sherlock, S., 1954. Intrasplenic pressure as index of portal venous pressure, *Lancet* **1**: 1325–1327.

Bibawi, E., Eldeeb, A. A., and Mahfouz, M. M., 1955. The portal circulation in hepatic fibrosis associated with bilharziasis, *Am. J. Trop. Med. & Hyg.* **4**: 913–922.

Dreyer, B., and Budtz-Olsen, O. E., 1952. Splenic venography. Demonstration of the portal circulation with diodone, *Lancet* **1:** 530–531.

Hunt, A. H., and Whittard, B. R., 1954. Thrombosis of the portal vein in cirrhosis hepatis, *Lancet* **1:** 281–284.

Lemaire, A., and Housset, E., 1955. La mesure de la pression portale par ponction du foie, *Presse Médicale* **36:** 1063–1064.

Paton, A., Reynolds, T. B., and Sherlock, S., 1953. Assessment of portal venous hypertension by catheterisation of hepatic vein, *Lancet* **1:** 918–921.

Walker, R. M., 1952. Pathology and treatment of portal hypertension, *Lancet* **1:** 729–733.

MOSQUITOES ASSOCIATED WITH SYLVAN YELLOW FEVER NEAR ALMIRANTE, PANAMA

HAROLD TRAPIDO[1] AND PEDRO GALINDO

Gorgas Memorial Laboratory, Panama, R. de P.

INTRODUCTION

This paper continues the reports we have been making on the forest mosquito fauna associated with the appearance of sylvan yellow fever in Panama. The sequence of yellow fever cases recognized in Panama from 1948 to 1952 has been reviewed by Elton (1952). The present study was undertaken following the histopathological diagnosis of a fatal case of yellow fever contracted near Almirante, Panama, in April 1951.

In February 1951 a group of men were engaged by the "Corporación de Ingeniería" under contract to the government of Panama to survey the route for a projected road from Almirante, a town on the Bahia de Almirante which opens into the Caribbean Sea some 20 miles southeast of the Costa Rican border, to Boquete, a village set in a mountain valley on the southeast side of the Volcan de Chiriqui at an elevation of about 4,000 feet. The air line distance between these two places is only about 40 miles, but the intervening country is composed of a series of mountain ridges so that the length of the projected road was about 100 miles. The slopes of this portion of the Isthmus of Panama facing the Caribbean are covered by tropical rain-forest. While there are scattered groups of Indians living in the region, the route of the proposed road was uninhabited virgin forest only occasionally visited by hunters. It is about ten days by foot from Boquete to Almirante.

On February 7th the engineer in charge of the survey, together with three other men, set out from Boquete to make a preliminary reconnaissance of the terrain between Boquete and Almirante. About a week later, while in the vicinity of a mountain locally known as Cerro Cuna, and at an elevation of about 1,000 feet, the field party came across several dead spider monkeys in the forest. Because of the remoteness of the place where these monkeys were found, and the delay in receiving the report, no investigation of the possibility that the monkey mortality was due to yellow fever was made.

The actual transit survey was begun from Almirante later in February, and by the first week in April had progressed 11.6 kilometers into the forest. Here a clearing was made and a field camp consisting of several palm-thatched "ranchos" was established at an elevation of 625 feet. About thirty men were based here engaged in clearing the path of the transit line and in surveying. Every second Friday afternoon the men walked into Almirante to be paid, returning to the field camp on Sunday afternoon. The second weekend in April, however, the men remained in town until Monday morning the 9th to receive vaccinations for typhoid and yellow fever at the Chiriqui Land Company Hospital there. They

[1] Present address: The Rockefeller Foundation, 49 West 49th Street, New York, N. Y.

returned on foot to the field camp on the afternoon of the same day. That night several of the men complained of a mild malaise—presumably a reaction to the typhoid vaccine. By Tuesday night, one of the men was seriously ill, and on Wednesday he was brought into the hospital at Almirante. The patient developed symptoms of yellow fever and died the following Sunday, April 15th. The clinical diagnosis was confirmed at autopsy and by histopathological examination of liver tissue. As the incubation period of yellow fever in man is generally accepted to be three to six days the infection must have been contracted only very shortly before vaccination. Dr. Gustav Engler, Medical Superintendent of the Almirante Hospital, has called to our attention two other recovered cases admitted about the same time which might be considered suspicious for yellow fever. These could not be confirmed by antibody tests as one had a history of having been vaccinated five months previously, and the other was vaccinated after his clinical episode before it was realized his illness might have been yellow fever. The possibility that yellow fever was contracted after vaccination cannot be excluded in light of the subsequent experience of Trejos and Romero (1954) in Costa Rica.

The circumstances under which yellow fever was contracted in the fatal case here reported were typical of the ecology of sylvan yellow fever. The patient had been a rodman with the survey crew. His duties required him to stand still in the lane freshly cleared through the forest. We know that under these conditions the predominantly arboreal mosquitoes attack at ground level.

On May 14, 1951 we went to Almirante to explore the possibility of making a long-term study of the forest mosquito fauna in the immediate area where yellow fever had been contracted. We found that the road survey had been abandoned, but that the palm-thatched shelters in the forest clearing at the survey base camp would be available to us for the establishment of a semi-permanent field camp. Basic supplies were brought into the field camp by mule. Arrangements were made for the simultaneous collection of the mosquitoes attacking human subjects on the ground and in the forest canopy on four days a week, and for the weekly shipment of the mosquito collections from Almirante to the Gorgas Memorial Laboratory in Panama City by air. Four trees were selected for collecting stations. Crossbars were nailed to the trees and platforms constructed in the crotches of the branches. The trees were all located within a half-mile radius of the field camp. The heights of the platforms above the ground were 48 feet, 39 feet, 54 feet and 36 feet. In October 1951 the tree with the 48 foot platform was blown down in a storm and another tree substituted. The replacement platform was 52 feet high. The four stations were located at elevations between 400 and 625 feet above sea-level. Collections were made on one day of each week at each station, with one man acting as both bait and collector on the ground at the foot of the tree station, and another on the platform in the canopy. This routine was followed for a two-year period from May 1951 through April 1953. Collections of all mosquitoes approaching to bite were made between 9:00 A.M. to 3:00 P.M. To obtain, also, some measure of the crepuscular and nocturnal mosquito populations, collections were made from 6:00 P.M. to 10:00 P.M. one evening each week, but only during a part of the study. The general aspect of the study area is shown in Figures 1 and 2.

FIG. 1. The clearing used as a base camp for the two year study of the sylvan mosquito fauna near Almirante, Panama. The density of the vegetation in this region of tropical rainforest is apparent.

FIG. 2. An aerial view of the unbroken virgin tropical rain-forest inland from Almirante, Panama, where the study reported here was carried out. The Bahia de Almirante may be seen in the distance; the river in the foreground and to the right is the Rio Changuinola. The forest tree stations were located on the ridge in the center of the photograph.

THE ENVIRONMENT

Rainfall. Directly behind Almirante there rises a series of complex ridges culminating in the highest point in Panama, the summit of the Volcan de Chiriqui with an elevation of 11,410 feet. There is thus extreme uplifting of the air mass of the moist northeasterly trade winds moving inland from the Caribbean Sea. In consequence heavy rainfall is experienced throughout the year. Table 1 gives rainfall figures based on the monthly averages of four stations maintained by the Chiriqui Land Company at Changuinola and vicinity for the years 1951 to 1953, the averages for these three years and the averages for a nine-year period. The Changuinola rain gauges are about 15 miles north of the location of the tree stations and at a lower elevation. While the day to day rainfall recorded at these gauges is no doubt different from that which falls at the forest tree stations, the annual distribution is much the same. These data serve to illustrate the lack of any pronounced annual dry season. On the Pacific side of Panama and the Atlantic side in the vicinity of the Canal Zone and the region to the east, where the mountain ridges are low, a distinct dry season occurs during the low-sun period from January through April. The local orographic situation at Almirante modifies this rainfall pattern. It will be seen from the three and nine-year summaries that on the average it is September which has the least rainfall, although there is no really "dry" month.

Vegetation. These rainfall data, together with the temperature data given below, meet the requirements of the "*Af*" or "tropical rain-forest climate" as

TABLE 1

Rainfall at Changuinola and vicinity

(In inches)

	1951	1952	1953	Three year average (1951–53)	Nine year average (1945–53)
January	6.39	13.83	9.75	9.99	8.23
February	19.81	3.70	7.57	10.36	7.51
March	5.44	2.24	3.15	3.61	4.81
April	5.96	5.97	3.31	5.08	6.03
May	5.43	3.85	7.08	5.45	10.34
June	10.45	3.57	7.02	7.01	10.08
July	16.50	8.23	11.42	12.05	11.64
August	12.28	2.62	17.20	10.70	8.36
September	4.65	3.58	1.92	3.38	3.82
October	6.29	13.46	5.83	8.53	5.97
November	7.60	4.41	15.30	9.10	11.02
December	7.00	12.96	9.93	9.96	12.33
Total	107.80	78.42	99.48	95.22	100.14

defined in the Köppen climatic system.[2] Thus, wherever either cultivation by man or special soil conditions have not intervened, the region is clothed in true tropical rain-forest. Such forest is characterized by a dense evergreen broad-leaved canopy, through which very little sunlight penetrates to the forest floor. The ground, therefore, is singularly free of annual herbaceous vegetation except where there may be a break in the canopy such as that caused by a large fallen tree, or along the margins of water courses cutting through the forest.

Climate and microclimate. The ultimate assessment of the ability of a region to maintain yellow fever transmission depends as much on the accurate and detailed measurement and evaluation of the microclimate as it does on the presence of vector and host. In fact, the presence of these also will have been determined in considerable measure by the elements of the climate. Thus we took opportunity at the Almirante stations to record for a typical sylvan yellow fever situation in well-developed tropical rain-forest the details of the climate and microclimate for the period of a year.

The climate and microclimate of an area of tropical rain-forest in Colombia in relation to captures of forest mosquitoes have been studied by Bates (1944 and 1945). Allee (1926) has also given some information on microclimatic differences between the forest floor and the canopy in terms of temperature, evaporation and light for a short period at Barro Colorado Island in the Canal Zone. At the Almirante forest stations we operated a pair of Bendix-Friez hygrothermographs for a fifteen-month period. One instrument was placed three feet above the ground and the other on a platform 40 feet high in the canopy. As it was necessary to protect the instruments from the elements in conventional louvered

[2] A brief exposition of the Köppen world climatic system in English will be found in Trewartha, 1954.

weather shelters, the readings are less extreme than those which would be gotten in the open. Free-flying insects in the sunlight of the canopy would experience higher temperatures and lower humidities than those recorded by the sheltered hygrothermograph. Nevertheless the differences in temperature and humidity conditions near the ground and in the canopy of a tropical rain-forest are nicely illustrated by months for a year's period in Figure 3. The monthly mean figures for temperature and relative humidity are based on bi-hourly readings from the tracings of the recording instruments. The monthly maxima and minima are averages calculated from the daily maxima and minima.

Since we unfortunately did not operate a rain gauge at the forest tree station where the hygrothermographs were located, we cannot closely relate rainfall to temperature and humidity conditions. This can be done in a general way, however, by using the rainfall figures from Changuinola.

Annual temperature cycle. For the annual march of temperatures we may refer to the monthly means near the ground. (See Figure 3.) The lowest mean monthly ground temperature, 71.7°F., was in January; the highest, 76.6°F. was in May. The extreme difference during the year was thus but 4.9°F. In terms of the environment experienced by the diurnal canopy mosquitoes the maximum figures are more significant. The variation during the year is not, however, very much greater, though the readings are, of course, in a higher range. The lowest maximum figure was that for January, 75.6°F., the highest maximum in September, 81.0°F.; the difference being 5.4°F. As the station was at about 9°15′N., the effect of latitude is evident but not extreme. The highest temperatures are not in July or August as might be expected from latitude effect alone, since the high rainfall at this time of year exercises a cooling effect.

Comparison of temperatures near the ground and in the canopy. The differences in maximum, mean and minimum temperatures near the ground and in the canopy may also be seen in Figure 3. For the year, the magnitude of the difference in temperatures near the ground and in the canopy was 1.6°F. for the maxima, 1.0°F. for the means, and 0.6°F. for the minima. The greater difference in the case of the maxima is no doubt due to the greater exposure of the canopy instrument shelter to sunlight during the day. The lesser difference in minimum figures is due to the fact that at night, when the minimum temperature conditions are experienced, there is less temperature gradient between the air near the ground and that in the canopy. From the point of view of the diurnal mosquitoes of special interest to us, the maximum temperature figures are the significant ones. The 1.6°F. difference recorded for the daily maxima under sheltered conditions would have been much greater had the recording instruments been unshaded; for then the canopy instrument would have been subject to direct sunlight a good deal of the time, while the instrument near the ground would have been struck only by occasional beams of sunlight penetrating to the forest floor.

Annual relative humidity cycle. Month-by-month differences in relative humidity are less pronounced than those shown by the temperature record. The high for the year, recorded in December, may be related to the high rainfall at

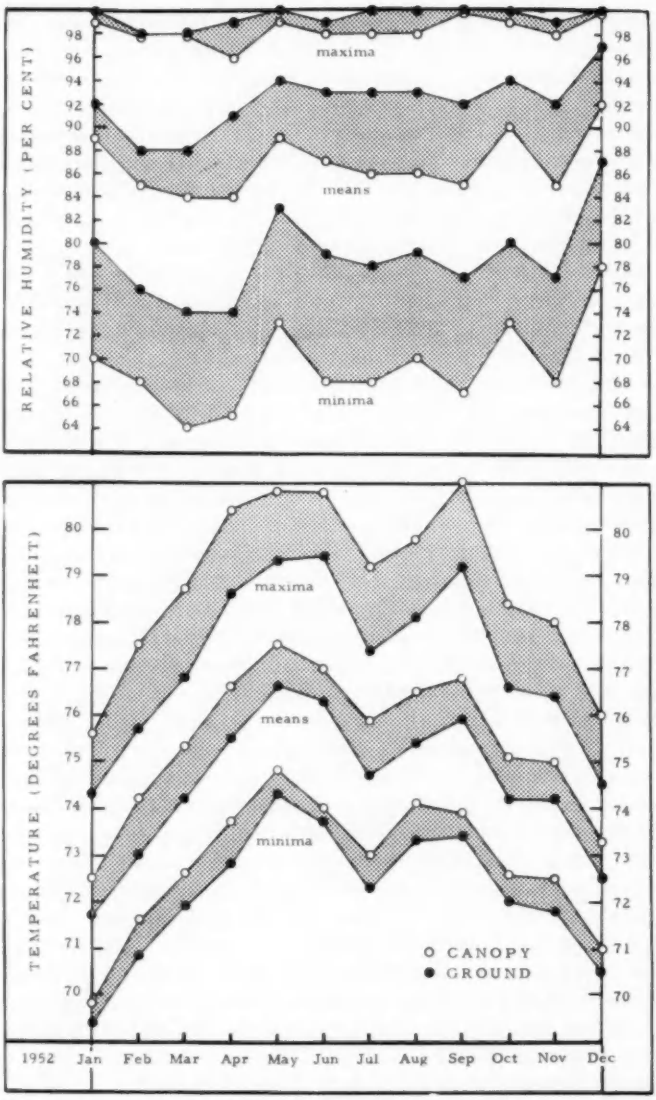


FIG. 3. Temperature and relative humidity during a year period at ground level and in the canopy of the tropical rain-forest near Almirante, Panama.

that time of year. Most striking is the height of the humidity level maintained in this tropical rain-forest. The monthly average of the mean relative humidities near the ground ranged between 88 and 97 per cent. The lowest relative humidity in the canopy was 64 per cent in March. The maximum relative humidity was

either at or near saturation throughout the year both at ground level and in the canopy.

Comparison of relative humidities near the ground and in the canopy. The greatest differences in relative humidity readings at ground level and in the canopy appear in the averages determined from the daily minima which occur during the daylight hours when most canopy mosquitoes are active. The average difference in the minima for the year period is 9.3 per cent. The differences in the maxima which occur at night are very slight, the average difference for the year being but 1.2 per cent. It has been suggested that to maintain canopy-inhabiting diurnal *Haemagogus* mosquitoes in captivity it is desirable to keep them at lower humidity levels than those suitable for such crepuscular or night-flying mosquitoes as the anophelines. This has not been our experience with two arboreal mosquitoes, *Haemagogus equinus* and *Sabethes chloropterus*, which we have established in laboratory colonies. The difficulty in the laboratory has been that of maintaining sufficiently high humidity to prevent excessive mortality of adults. While there is a substantial spread between the canopy and ground relative humidity readings as shown by the minima in Figure 3, this spread occurs only during the sunlight hours of the day when, it is true, the canopy mosquitoes are active. But during the night, and during the periods of rain, mist and heavy overcast which account for a good part of the day in the tropical rainforest climate in which these mosquitoes occur, there is very little difference between the relative humidity at canopy and ground level. Even during hours of sunshine, unless the canopy mosquitoes are seeking food or are mating, they probably spend much time at rest in the humid microclimate associated with the surface of leaves and branches. The microclimate of these resting places is at least as important as that which is encountered when they are in flight.

Mosquito captures

Summaries of the diurnal and evening mosquito captures made during the two years of this study are given in Tables 2 and 3. Table 2 also shows for each species the proportion of the catch taken in each yearly period, corrected for the number of man hours of collecting effort. The distribution of the diurnal catch between the canopy and the ground stations is shown in Table 4, and for the evening catch in Table 3. As the evening collections were carried on only once a week during 13 months no breakdown of the catch into yearly periods is made. In all the tabulations the figures refer to numbers of female mosquitoes only. Males which incidentally approached the human baits were not included in the workup of the data.

Haemagogus. The total numbers of *Haemagogus equinus* and *H. spegazzinii falco* were approximately the same, but the proportions taken during each yearly period were different. The *equinus* were taken in approximately equal numbers during each yearly period while the *spegazzinii falco* were about twice as abundant in the first year as in the second. *Haemagogus lucifer* was somewhat less common than the other two species of the genus and the numbers taken during each of the two yearly periods were fairly even. The fact that the numbers of *spegazzinii falco* approximate those of *equinus* we consider significantly related to the climate

TABLE 2

Summary of diurnal mosquito captures at tree stations near Almirante during a two-year period

	May 1951–April 1952	May 1952–April 1953	Two-year total	[1]Per cent captured during 1951–52	Per cent captured during 1952–53
Number of man hours collecting	2,036	1,865	3,901		
Tribe Culicini					
Haemagogus equinus	569	582	1,151	47	53
Haemagogus lucifer	576	407	983	56	44
Haemagogus spegazzinii falco	794	347	1,141	68	32
Total *Haemagogus*	1,939	1,336	3,275	57	43
Aedes (Finlaya) leucocelaenus clarki	318	543	861	35	65
Aedes (Finlaya) leucotaeniatus	138	159	297	44	56
Aedes (Finlaya) terrens	40	11	51	77	23
Aedes (Howardina) quadrivittatus	9	19	28	30	70
Aedes (Howardina) septemstriatus	25	7	32	76	24
Aedes (Howardina) sexlineatus	55	79	134	39	61
Aedes (Ochlerotatus) angustivittatus	49	405	454	10	90
Aedes (Ochlerotatus) fulvus	1	4	5	19	81
Aedes (Ochlerotatus) hastatus	0	18	18	0	100
Aedes (Ochlerotatus) serratus group	253	1,518	1,771	13	87
Psorophora (Janthinosoma) ferox	99	2,352	2,451	4	96
Psorophora (Janthinosoma) lutzii	182	735	917	19	81
Psorophora (Grabhamia) cingulata	16	17	33	46	54
Mansonia (Mansonia) indubitans	2	9	11	17	83
Mansonia (Mansonia) titillans	27	6	33	81	19
Mansonia (Rhynchotaenia) arribalzagae	79	11	90	87	13
Mansonia (Rhynchotaenia) nigricans	2	0	2	100	0
Mansonia (Rhynchotaenia) venezuelensis	15	49	64	22	78
Culex (Carrollia) secundus	3	4	7	42	58
Culex (Culex) sp.	4	148	152	3	97
Culex (Melanoconion) sp.	2	23	25	8	92
Culex (Microculex) sp.	0	2	2	0	100
Total Culicini	3,258	7,455	10,713	29	71
Tribe Sabethini					
Trichoprosopon (Trichoprosopon) digitatum	102	46	148	67	33
Trichoprosopon (Ctenogoeldia) magnus	484	184	668	71	29
Trichoprosopon (Isogoeldia) espini	95	77	172	53	47
Trichoprosopon (Rhunchomyia) lampropus	13	5	18	70	30
Trichoprosopon (Rhunchomyia) leucopus	160	289	449	34	66
Trichoprosopon (Rhunchomyia) longipes	36	19	55	63	37
Trichoprosopon (Rhunchomyia) rapax	1	5	6	16	84
Trichoprosopon sp.	20	8	28	69	31
Wyeomyia (Wyeomyia) abebela	1	0	1	100	0
Wyeomyia (Wyeomyia) celaenocephala	50	3	53	94	6
Wyeomyia (Wyeomyia) hemisagnosta	2	0	2	100	0
Wyeomyia (Wyeomyia) mitchelli	2	0	2	100	0
Wyeomyia (Wyeomyia) nigritubus	4	0	4	100	0
Wyeomyia (Wyeomyia) quasiluteoventralis	1	0	1	100	0
Wyeomyia (Wyeomyia) scotinomus	24	0	24	100	0
Wyeomyia (Wyeomyia) sp.	10,230	11,393	21,623	45	55

TABLE 2—*Continued*

	May 1951–April 1952	May 1952–April 1953	Two-year total	[1]Per cent captured during 1951–52	Per cent captured during 1952–53
Number of man hours collecting	2,036	1,865	3,901		
Wyeomyia (Dendromyia) aporonoma	6	3	9	64	36
Wyeomyia (Dendromyia) circumcincta	2	0	2	100	0
Wyeomyia (Dendromyia) jocosa	28	0	28	100	0
Wyeomyia (Dendromyia) sp.	4,624	2,912	7,536	59	41
Wyeomyia (Davismyia) arborea	14	10	24	56	44
Limatus asulleptus	165	314	479	33	67
Limatus durhami	12	7	19	61	39
Sabethes (Sabethes) cyaneus	201	120	321	61	39
Sabethes (Sabethes) tarsopus	90	141	231	37	63
Sabethes (Sabethoides) chloropterus	1,736	1,879	3,615	46	54
Total Sabethini	18,103	17,415	35,518	49	51
Tribe Anophelini					
Anopheles (Anopheles) apicimacula	6	13	19	29	71
Anopheles (Anopheles) eiseni	7	4	11	62	38
Anopheles (Anopheles) punctimacula	1	3	4	24	76
Anopheles (Nyssorhynchus) albimanus	0	1	1	0	100
Anopheles (Kerteszia) neivai	139	15	154	89	11
Chagasia bathanus	14	8	22	62	38
Total Anophelini	167	44	211	78	22
Total all species	21,528	24,914	46,442	44	56

[1] Percentages corrected for differences in number of man hours of collecting during each year period.

and forest type in which these collections were made. In our previous studies in central Panama and the Canal Zone (Galindo *et al.*, 1950) and throughout the length of the Pacific side of Panama and adjacent Costa Rica (Trapido *et al.*, 1955) we had found *equinus* to be approximately six to eight times as abundant as *spegazzinii falco*. In these previous surveys the numbers of *spegazzinii falco* were found to more nearly approach the numbers of *equinus* in only two circumstances. In the survey reported in 1950 one group of stations near Buena Vista was operated only from October to the beginning of January in an attempt to recover virus in a forest where a fatal case of yellow fever had been contracted. In this instance the number of *equinus* taken was less than twice the number of *spegazzinii falco*. In the study reported in 1955 only slightly less than half as many *spegazzinii falco* as *equinus* were taken at the stations at Tucué. The Buena Vista stations, while in an area of tropical semideciduous forest, were operated only during the fall peak rain period; the Tucué stations were just over the continental divide on the Caribbean slope in an area with vegetation approaching the tropical rain-forest type. It seems clear that populations of *spegazzinii falco* develop best in tropical rain-forest or under climatic conditions approaching those which support forest of this type. Under these conditions

TABLE 3

Summary of evening mosquito captures at tree stations near Almirante during the period July 1951 to May 1953
(Collections made from 6–10 P.M.)

	Total	[1]Per cent captured	
Number of man hours collecting	295	Canopy	Ground
Tribe Culicini			
Haemagogus equinus	2	100.0	0.0
Haemagogus lucifer	1	100.0	0.0
Haemagogus spegazzinii falco	3	100.0	0.0
Total *Haemagogus*	6	100.0	0.0
Aedes (Finlaya) leucocelaenus clarki	2	100.0	0.0
Aedes (Finlaya) leucotaeniatus	2	100.0	0.0
Aedes (Finlaya) fluviatilis	1	0.0	100.0
Aedes (Howardina) sexlineatus	4	100.0	0.0
Aedes (Ochlerotatus) angustivittatus	22	4.4	95.6
Aedes (Ochlerotatus) serratus group	97	14.2	85.8
Psorophora (Janthinosoma) ferox	16	17.1	82.9
Psorophora (Janthinosoma) lutzii	6	47.6	52.4
Psorophora (Grabhamia) cingulata	118	11.4	88.6
Mansonia (Mansonia) indubitans	12	83.3	16.7
Mansonia (Mansonia) titillans	3	47.6	52.4
Mansonia (Rhynchotaenia) arribalzagae	10	51.4	48.6
Mansonia (Rhynchotaenia) venezuelensis	119	33.8	66.2
Culex (Culex) inflictus	1	100.0	0.0
Culex (Culex) sp.	467	70.6	29.4
Culex (Melanoconion) menytes	120	80.3	19.7
Culex (Melanoconion) taeniopus	10	31.2	68.8
Culex (Melanoconion) sp.	206	61.9	38.1
Culex (Microculex) sp.	5	100.0	0.0
Culex sp.	36	83.3	16.7
Orthopodomyia fascipes	2	100.0	0.0
Total *Culicini*	1,265	49.8	50.2
Tribe Sabethini			
Trichoprosopon (Ctenogoeldia) magnus	2	100.0	0.0
Trichoprosopon (Rhunchomyia) leucopus	1	100.0	0.0
Wyeomyia (Wyeomyia) mitchelli	1	100.0	0.0
Wyeomyia (Wyeomyia) scotinomus	2	100.0	0.0
Wyeomyia (Wyeomyia) sp.	14	85.4	14.6
Sabethes (Sabethoides) chloropterus	4	100.0	0.0
Total *Sabethini*	24	91.2	8.8
Tribe Anophelini			
Anopheles (Anopheles) apicimacula	37	11.1	88.9
Anopheles (Anopheles) eiseni	1	0.0	100.0
Anopheles (Kerteszia) neivai	120	100.0	0.0
Anopheles sp.	11	100.0	0.0
Chagasia bathanus	266	99.2	0.8
Total *Anophelini*	435	85.5	14.5
Total all species	1,724	57.8	42.2

[1] Percentages corrected for differences in number of man hours collecting on the ground and in the canopy.

populations of *equinus* are smaller than they are in the more open tropical deciduous forests associated with a rainfall pattern exhibiting a marked dry season such as that experienced on the Pacific side of Panama.

The numbers of *Haemagogus* taken in the evening collections as shown in Table 3 were negligible.

TABLE 4

Vertical distribution of diurnal forest mosquitoes
Tropical rain-forest at Almirante compared with tropical deciduous forest of the Pacific slope of Panama

Species	Almirante (Tropical rain-forest)			Pacific Slope[1] (Tropical deciduous forest)		
	Per cent captured		Number taken	Per cent captured		Number taken
	Canopy	Ground		Canopy	Ground	
Haemagogus equinus	97.8	2.2	1,151	69.1	30.9	6,431
Haemagogus lucifer	98.7	1.3	983	67.1	32.9	3,351
Haemagogus spegazzinii falco	99.6	0.4	1,141	80.7	19.3	743
Aedes leucocelaenus clarki	94.1	5.9	861	65.7	34.3	966
Aedes leucotaeniatus	39.7	60.3	297	40.1	59.9	434
Aedes terrens	10.0	90.0	51	17.4	82.6	1,533
Aedes quadrivittatus	96.5	3.5	28	74.3	25.7	2,227
Aedes septemstriatus	9.2	90.8	32	14.3	85.7	46
Aedes sezlineatus	96.4	3.6	134	10.9	89.1	16
Aedes angustivittatus	2.2	97.8	454	16.7	83.3	804
Aedes hastatus	0.0	100.0	18			
Aedes serratus group	2.0	98.0	1,771	9.8	90.2	2,476
Psorophora feroz	10.9	89.1	2,451	23.2	76.8	6,180
Psorophora lutzii	38.3	61.7	917	23.0	77.0	867
Psorophora cingulata	0.0	100.0	33	19.7	80.3	173
Mansonia titillans	18.4	81.6	33	8.7	91.3	877
Mansonia arribalzagae	2.2	97.8	90			
Mansonia venezuelensis	3.1	96.9	64	24.1	75.9	293
Trichoprosopon digitatum	2.0	98.0	148	15.9	84.1	71
Trichoprosopon magnus	87.5	12.5	668	73.0	27.0	8,257
Trichoprosopon espini	82.7	17.3	172	60.1	39.9	84
Trichoprosopon lampropus	0.0	100.0	18			
Trichoprosopon leucopus	2.4	97.6	449	0.0	100.0	19
Trichoprosopon longipes	0.0	100.0	55	20.8	79.2	2,049
Wyeomyia celaenocephala	9.6	90.4	53			
Wyeomyia scotinomus	17.1	82.9	24			
Wyeomyia jocosa	0.0	100.0	28	28.3	71.7	261
Wyeomyia arborea	95.9	4.1	24	100.0	0.0	20
Limatus asulleptus	0.2	99.8	479	2.8	97.2	195
Limatus durhami	5.2	94.8	19	14.6	85.4	120
Sabethes cyaneus	84.5	15.5	321	87.4	12.6	348
Sabethes tarsopus	99.2	0.8	231	87.4	12.6	655
Sabethes chloropterus	98.1	1.9	3,615	75.9	24.1	3,170
Anopheles apicimacula	0.0	100.0	19	10.3	89.7	226
Anopheles neivai	70.9	29.1	154	40.3	59.7	183
Chagasia bathanus	95.6	4.4	22	85.8	14.2	26
Total			17,008			43,101

[1] Data from Trapido, Galindo and Carpenter, 1955.

Two taxonomic points with regard to the *Haemagogus* at the Almirante stations should be noted. *H. lucifer* is replaced in nearby Costa Rica and in Nicaragua by *Haemagogus iridicolor*, the females of which are indistinguishable from *lucifer*. *H. iridicolor* has also been taken by us along the shores of the Bahia de Almirante in Panama. But a series of males reared from eggs of females taken at the inland forest tree stations were all *lucifer*, and all males taken there were also *lucifer*, so we believe we are correct in assuming that the species in that area is *lucifer*. At the time this study was made *Haemagogus mesodentatus* was known only from the type description based on material from San José, Costa Rica (Komp and Kumm, 1938) and notation of its presence in El Salvador (Kumm and Zuñiga, 1942). The type description did not mention a critical character of the female, namely the presence of a silvery white band of scales at the tip of the mid and hind femora, which is also a primary field character for identifying *H. equinus*; no other Panamanian *Haemagogus* was known to be thus marked at the time. In a substantial topotype series of *mesodentatus* we subsequently obtained at San José we found the white "knee spots" to be present in females as they are in *equinus*. We have also been able to determine more recently that *H. mesodentatus* ranges southeast into Panama in the forests of Bocas del Toro Province between Caldera and Chiriquicito. In unrubbed specimens the females of *mesodentatus* may be readily separated from *equinus* by another character, the silvery grey scales of the mid-tarsi. At the time of the Almirante study we did not suspect the presence of *mesodentatus* in Panama and this was not a character we checked in routine identification work. But after we became familiar with *mesodentatus* we found its distribution to be quite local in the southern part of its range in Costa Rica and Nicaragua. This is a species whose populations in our experience reach their full development far to the northwest in Guatemala and Mexico. Thus it does not follow from the fact that it is present in the area between Caldera and Chiriquicito that it was also present at the forest stations near Almirante. On recently checking a representative sample of white kneed *Haemagogus* which were saved from the Almirante collections we have found all to be *equinus*. While it remains a possibility that *mesodentatus* as well as *equinus* was present at the Almirante forest stations we are inclined to think that we did not overlook it there.

Aedes. Four species of the subgenus *Finlaya* were taken at the Almirante forest stations. Most abundant of these was *Aedes leucocelaenus clarki*. This is a species of particular interest since it, like *H. spegazzinii falco*, has been proven to be a vector of sylvan yellow fever in South America (See Whitman, 1951). In previous surveys in Panama this species had been about as abundant as *H. spegazzinii falco*, but at Almirante it was outnumbered by the latter. As in the case of *spegazzinii falco*, approximately two-thirds of the specimens were taken in one year and one-third in the other. But, curiously, the greater number was taken in the 1952–53 year period while *spegazzinii falco* was more abundant in 1951–52. We have no explanation of why this should be so. This shows, however, that the factors which govern the production of tree-hole-breeding mosquitoes are complex. A rainfall pattern which yields a high production of one tree-hole-

breeding species in a particular year does not necessarily affect other tree-hole-breeding species in the same way. *Aedes leucotaeniatus*, which is very closely related to *leucocelaenus clarki*, was much less abundant than the latter species. What currently goes under the name *Aedes terrens* is more than one species. The lowland form does not attack man readily and was not very common in the Almirante collections. Although the numbers of *Finlaya* taken in the evening collections was insignificant, it was in the evening that one specimen of *Aedes fluviatilis* was taken.

Three species taken of the *Aedes* subgenus *Howardina* were *quadrivittatus*, *septemstriatus* and *sexlineatus*. These also breed in water containers; *septemstriatus* in tree holes and the other two species in bromeliads. They were not abundant in the collections attacking man. Only *sexlineatus* appeared in the evening collections.

Four ground pool breeders of the subgenus *Ochlerotatus* were taken: *angustivittatus*, *fulvus*, *hastatus* and the *serratus* group. The mosquitoes of the *serratus* group were not identifiable as to species on the basis of the females taken attacking man. Light traps operated at the Almirante stations during a portion of this study took males of several species in this group. They will be dealt with in another paper specifically treating taxonomic and distributional problems. Only the *serratus* group and *angustivittatus* were common. All four species illustrate how irregular the hatches of these mosquitoes may be in nature. The bulk of the collections appeared in the 1952–53 year period. Only the commoner forms, *angustivittatus* and the *serratus* group were taken in the evening collections.

Psorophora. *Psorophora lutzii* and *ferox* were both abundant, with about three times as many *ferox* as *lutzii* being taken. These ground-pool-breeding species were much more abundant in the second year of the study than they were in the first, as was the case with the ground-pool-breeding *Aedes*. Both species are diurnal as may be seen by comparison of the numbers caught during the day in Table 2 with the numbers caught at night given in Table 3. *Psorophora cingulata*, on the other hand, was uncommon in the diurnal collections but much outnumbered the other two *Psorophora* in the evening collections. This species is clearly crepuscular or nocturnal unlike *lutzii* and *ferox*.

Mansonia. The *Mansonia* mosquitoes which are primarily crepuscular and nocturnal, and breed attached to the roots of vegetation in swamps were not very abundant at the Almirante forest stations which were on elevated slopes. Five species of two subgenera were taken: *indubitans* and *titillans* of the subgenus *Mansonia*, and *arribalzagae*, *nigricans* and *venezuelensis* of the subgenus *Rhynchotaenia*. *M. venezuelensis*, in the evening collections, was the commonest species.

Culex. As the identification of the *Culex* depends primarily on characters of the male terminalia few of these were determined to species. Only one form was identified to species in the diurnal collections, *Culex secundus*. In the evening collections the three species *inflictus*, *menytes* and *taeniopus* were determined. It was not previously realized that the species *menytes* and *taeniopus* bite man.

Trichoprosopon. *Trichoprosopon* of four subgenera and seven species were taken. In order of greatest to least abundance in the diurnal catch the species

were *magnus*, *leucopus*, *espini*, *digitatum*, *longipes*, *lampropus* and *rapax*. Only the first four species were moderately abundant. The relatively large numbers of *Trichoprosopon magnus*, a species breeding in a *Calathea* characteristic of swampy situations, is explained by the presence of a small open spring-fed bog in the forest near the tree stations. This species is of some interest in the epidemiology of sylvan yellow fever as it was the commonest arboreal mosquito present during an epizootic of the disease investigated on the north coast of Honduras in 1954 (Trapido and Galindo, 1955). *Trichoprosopon* were rare in the evening collections, only two specimens of *magnus* and one of *leucopus* being taken.

Wyeomyia. The identification of female *Wyeomyia* to species is difficult or impossible if the scales are rubbed; thus these mosquitoes were for the most part determined only to subgenus. Of the few which were identified further, there appeared 11 species of the three subgenera *Wyeomyia*, *Dendromyia* and *Davismyia*. Of interest is the large number of this genus in comparison with the total catch. At the Almirante tropical rain-forest stations the *Wyeomyia* comprised more than 60 per cent of the total catch, while in the survey made in the deciduous tropical forest of central Panama (Galindo *et al.*, 1950) they made up only about three per cent of the catch. This may be explained by the fact that most mosquitoes of this genus breed in water-holding plants, primarily epiphytes, which are more abundant in the tropical rain-forest than in the tropical deciduous forest. The *Wyeomyia* are diurnal mosquitoes, and only insignificant numbers were taken in the evening collections.

Limatus. Two species of *Limatus* were present in the diurnal collections, *asulleptus* and *durhami*, with the former far more abundant than the latter.

Sabethes. *Sabethes chloropterus*, the most abundant representative of this genus at these stations as elsewhere in Panama, was fairly evenly distributed during the two years of the survey. Much less common were *Sabethes cyaneus* and *tarsopus*. Both these species demonstrated an irregular annual distribution, about two-thirds of the collections being taken in one year and one-third in the other. But as in the case of two other tree hole breeding species, *A. leucocelaenus clarki* and *H. spegazzinii falco*, *cyaneus* was more abundant in the first year while *tarsopus* was more abundant in the second year. This again illustrates how differently the same climate cycle may affect species with apparently similar breeding habits. The *Sabethes* are diurnal species and only insignificant numbers of the common *chloropterus* were taken in the evening.

Anophelini. There were five species of *Anopheles* in the collections: *apicimacula*, *eiseni*, *punctimacula*, *albimanus* and *neivai*. Only the bromeliad breeding *neivai* of the subgenus *Kerteszia* was present in substantial numbers either in the diurnal or evening collections. It is of interest that so large a number of this species was taken biting in the daytime collections, and also that the bulk of the daytime captures were made during the first year of the survey. In the traverse survey made from Caldera to Chiriquicito in 1955 another *Kerteszia* was taken, *Anopheles boliviensis*, which was previously known only from South America (see Lane, 1953). As we kept the *Kerteszia* specimens from the Almirante forest stations, it has been possible to check them to be sure we did not overlook *boliviensis*.

None were present in the Almirante collections. This serves to confirm our belief that the finding of *H. mesodentatus* on this same traverse survey between Caldera and Chiriquicito does not necessarily imply that this species was present at the Almirante forest stations also.

Fairly common in the evening collections was *Chagasia bathanus*. It was rare in the diurnal catches, and may properly be considered crepuscular or nocturnal as are the other anophelines in general.

Vertical stratification

The data from the Almirante stations, located as they were in virgin tropical rain-forest, are particularly valuable for illustrating how forest type affects vertical stratification of the mosquito fauna. The summary for the Almirante stations is given in Table 4, and for comparison there are included in this table the data from the series of stations operated in the tropical deciduous forest of the Pacific side of Panama during 1950, which were reported in a previous paper (Trapido *et al.*, 1955). The 1950 survey included one station, Tucué, which was just across the continental divide in the Caribbean drainage. But because of the relatively low elevation of the divide there the tropical rain-forest rainfall pattern was not as well developed as at the Almirante stations, and the forest was intermediate between the tropical-rain and tropical deciduous types. All the other Pacific side stations were located in either tropical deciduous or semi-deciduous forest. The data from the 1950 collections may thus be validly compared with those from Almirante to demonstrate the difference in the vertical stratification of the catch in different forest types. In the case of both sets of figures the percentages shown in Table 4 were obtained by making correction for the number of hours expended in collecting on the ground and in the canopy. Per cent captures in the canopy and on the ground were calculated only for those species of which more than 15 specimens were taken. In the case of the Almirante collections there were 36 species which met this requirement, while 31 of the same species appeared in the 1950 survey.

It has been shown by such authors as Bates (1944) and Haddow (1954) that the vertical stratification of the forest mosquito fauna shifts during different hours of the day and between wet and dry season. We believe the comparisons we here make between the stratification at the Almirante stations and those of the Pacific side of Panama to be valid for the following reasons. At both sets of stations the collections were made between 9 A.M. and 3 P.M. The Pacific side collections were made from June to December, the rainy season months, while the Almirante work was carried on throughout a two-year period. But since at Almirante there is no pronounced dry season, the collections were made under rainfall conditions in general similar to those of the rainy season of the Pacific slope. The collections were made over such long periods that effects produced by minor weather fluctuations would be smoothed out in the general averages.

The results from the Almirante stations differ in a number of particulars from those obtained in our first study of the forest mosquito fauna in central Panama and the Canal Zone done in 1949 (Galindo *et al.*, 1950). At that time, however,

we collected at platforms midway up the trees as well as in the canopy and on the ground. Under these conditions there was diversion of a portion of the catch, and the results may not be readily compared with those obtained at the stations at which only canopy and ground collections were made. Thus we will not attempt to make comparisons with these data.

The generalization which emerges from a study of the data in Table 4 is that species which are predominantly arboreal in tropical deciduous forest are more distinctly so in tropical rain-forest, while those predominantly taken at ground level in tropical deciduous forest show this tropism in more extreme fashion in the rain-forest. That is to say, in general, the stratification of the mosquitoes between canopy and ground is much more pronounced in rain-forest than it is in deciduous forest.

Arboreal species. Of the 31 species occurring at both sets of stations, 12 were taken in the canopy of the tropical deciduous forest more than 60 per cent of the time. The percentage of these taken in the canopy of the rain-forest was substantially higher in the case of ten of these 12 species. The two exceptions were *Wyeomyia arborea* and *Sabethes cyaneus*. In the case of the extremely arboreal *Wyeomyia arborea* (we named this species in recognition of this characteristic, Galindo *et al.*, 1951), 100 per cent were taken in the canopy of the deciduous forest so that a greater percentage could not possibly have been gotten in the canopy of the rain-forest. The numbers taken at both sets of stations were small, so that the percentage shown for the rain-forest stations (95.9) represents only a single specimen taken on the ground. This exception to the general rule given above is therefore not significant. *Sabethes cyaneus* thus remains as an exception to the generalization made above, although the percentage taken in the canopy of the deciduous forest was only slightly higher (2.9 %) than in the rain-forest. Striking increases in the percentages taken in the canopy are shown by the three species of *Haemagogus*, *Aedes leucocelaenus clarki* and *quadrivittatus*, *Trichoprosopon magnus* and *espini*, *Sabethes tarsopus* and *chloropterus*, and *Chagasia bathanus*. As an example of an extremely arboreal species we may take *H. spegazzinii falco*. Of 1,141 specimens taken, 99.6 per cent were in the canopy at the Almirante rain-forest stations while but 80.7 per cent of 743 specimens were collected in the canopy of the deciduous forest. The stratification of this species was also more marked at the Almirante stations than at two localities where it had been studied in Colombia (Bates, 1944) and Brazil (Causey and Dos Santos, 1949). The most extreme increase in preference for the arboreal habitat was shown by *H. lucifer* with 67.1 per cent in the canopy of the deciduous forest and 98.7 per cent arboreal in the rain-forest, an increase of 31.6 per cent.

These data nicely illustrate an important point in the epidemiology of sylvan yellow fever. In the virgin tropical rain-forest near Almirante the vector species of *Haemagogus* and *A. leucocelaenus* are so completely arboreal that human beings could probably pass through this forest in the presence of a raging epizootic among monkeys in the canopy, without danger of being infected on the ground. In tropical deciduous forest the vertical stratification of the mosquitoes is pronounced but much less so than in the case of rain-forest. Where the canopy of the

rain-forest is broken by clearing, or even the felling of a single large tree, the microclimate of the canopy is brought near the ground and there is real danger of being bitten by arboreal mosquitoes. It was a circumstance of this sort which produced the yellow fever case near Almirante. We routinely make use of this fact when engaged in rapid surveys of canopy mosquitoes. Where it is not feasible to climb into the canopy for collecting we fell several trees, letting the sunlight through to the forest floor. Samples of mosquitoes taken attacking in the fresh opening in the forest then include the arboreal species. This technique has been used for some years by our colleague, Dr. Jorge Boshell. In deciduous forest it is often not necessary to disturb the canopy to obtain a sample of the essentially arboreal mosquito fauna at ground level, though an exception to this generalization would be such a species as *Wyeomyia arborea*.

Of the mosquitoes taken in some numbers in the evening collections there were three predominantly arboreal species, *Culex menytes*, *Anopheles neivai* and *Chagasia bathanus*. As the gradients of light, temperature and humidity between canopy and ground are much less marked during the evening and early night hours during which these collections were made, there is indication that factors other than the microclimatic ones discussed earlier in this paper are involved. Some evidence of height orientation based on factors other than light has previously been suggested by the laboratory experiment of Bates (1947). Another factor which should be considered as concerned in determining vertical stratification is that of host preference. That is, a species with a strong preference for feeding on primate blood would need to seek out the forest canopy to find its host. The strong arboreal preference of the anophelines, *A. neivai* and *Ch. bathanus*, may be of significance in a disease problem other than yellow fever, *i.e.*, the transmission of the monkey malaria parasite, *Plasmodium brazilianum*, which occurs in forest primates in this area as well as in South America.

Ground level species. Sixteen of the diurnal species in the deciduous forest were taken more than 60 per cent of the time on the ground. Thirteen of these were taken with greater relative frequency on the ground than in the canopy under rain-forest conditions at Almirante. These species were *Aedes terrens*, *septemstriatus*, *angustivittatus* and the *serratus* group, *Psorophora ferox* and *cingulata*, *Mansonia venezuelensis*, *Trichoprosopon digitatum* and *longipes*, *Wyeomyia jocosa*, *Limatus asulleptus* and *durhami*, and *Anopheles apicimacula*. Exceptions were *Mansonia titillans* and *Trichoprosopon leucopus* of which slightly higher proportions were taken on the ground in the deciduous forest. But the numbers of the former (33) were small in the rain-forest and the numbers of the latter (19) were small in the deciduous forest, so that the comparisons probably are not very significant. Substantial numbers of *Psorophora lutzii* were taken in both types of forest, and the failure of this species to demonstrate increased ground-oriented stratification in the rain-forest is a plain exception to the generalization that stratification is more extreme in rain-forest. *Psorophora cingulata* which was more abundant in the evening than in the daytime collections showed strong preference for the ground in both circumstances.

Five species were taken at the Almirante rain-forest stations for which com-

parative stratification figures from the Pacific slope deciduous forest stations are not available. These species, *Aedes hastatus*, *Mansonia arribalzagae*, *Trichoprosopon lampropus*, *Wyeomyia celaenocephala* and *scotinomus*, were all preponderately present in the ground level collections.

Indifferent species. Only a single species, *Aedes leucotaeniatus*, appears to be relatively evenly distributed between the canopy and the ground level, though slightly favoring the ground. The proportions taken at canopy and ground level in both types of forest were almost exactly the same. As this species is very closely related to *A. leucocelaenus clarki* it is of interest to note how differently it stratifies in the forest.

Species showing inconsistent behavior. Two species demonstrate "switches" in their relative abundance in the canopy and near the ground in the two types of forest, *Aedes sexlineatus*, and *Anopheles neivai*. Both appeared predominantly in the canopy at the rain-forest stations and predominantly at ground level in the deciduous forest. The number of *A. sexlineatus* was small (16) in the deciduous forest and the ground preference shown in this habitat is probably not significant. The samples of *A. neivai* in both types of forest were large enough to have produced a consistent pattern. We have no ready explanation of why this species showed preference for the ground level in the deciduous forest and the canopy in the rain-forest. Its arboreal predominance in the daytime while marked was much exceeded by that shown in the evening collections in which 100 per cent of 120 specimens were taken in the canopy.

Insects other than mosquitoes. Other biting insects taken in forest canopy have been *Phlebotomus* sandflies and Tabanids. Our colleague, Dr. Graham Fairchild, has discussed the vertical stratification of the Tabanidae (Fairchild, 1953); and Fairchild and Hertig (1952) have noted the arboreal tendency of *Phlebotomus trapidoi* which was taken in numbers in the evening collections at Almirante.

SUMMARY

The clear cut nature of the vertical stratification of the mosquito fauna of the tropical rain-forest is well illustrated if we eliminate the one "indifferent" species and the two species which "switched" their distribution in the two forest types, and then enumerate the remaining 33 species in ten per cent groups. In the case of the 12 canopy mosquitoes nine species were 90 per cent or more, and three from 80 to 90 per cent in the canopy. Of the remaining 21 species 17 were 90 per cent or more at ground level, three from 80 to 90 per cent at ground level, and only one in the 60 to 70 per cent bracket.

Daily activity cycle

The daily activity cycle as we deal with it here concerns only activity as measured by mosquitoes approaching a bait to bite. This subject deserves careful study not only for itself but also for its importance in any basic understanding of the disease transmission cycles in which these mosquitoes may be involved. In the present fluid state of our knowledge of the dynamics of daily mosquito activity cycles any serious attempt to analyze the mass of data accumulated at

Almirante on a causal basis would require a lengthy and involved presentation. The difficulties inherent in the various methods of collecting the data and analyzing them, as well as those of interpreting the results, have recently been pointed out by Haddow (1954). As our purpose in this paper is to give the overall picture of the activity of the mosquito fauna of a rain-forest yellow fever area, we will confine ourselves to a general summary of the findings on the daily periodicity or "biting cycle."

Haddow (1954) has warned of the pitfalls in drawing conclusions with regard to biting cycles based on anything less than 24-hour catches. While his point is well taken, we are confident that such species as the *Haemagogus*, the *Aedes* of the subgenus *Finlaya*, the *Trichoprosopon* and *Sabethes* are distinctly diurnal. The several individuals of these species taken in the evening collections were taken before the hour of nightfall. This does not hold, of course, for such species as *Psorophora cingulata* and *Anopheles neivai* which were present in substantial numbers during the evening and early night as well as during the day. The diurnal collections were confined to the hours 9 A.M. to 3 P.M., as this period could be handled by a single crew of men, and since it was our empirical impression that these hours were the ones of greatest activity of the diurnal forest mosquitoes known or suspected to transmit yellow fever. The evening collections were made only incidentally, and at times irregularly, and they are therefore not suited to analysis of periodicity.

For the interpretation of daily biting cycles Haddow (1954) considers the geometric mean a better measure of central tendency than plain averages (arithmetic means), particularly when dealing with data drawn from a small series of days. This is because the results are "apt to be unduly influenced by extreme values, due to individual catches" (Haddow, 1954, p. 219). In the case of our data, however, we believe the averages to be meaningful since we are dealing with a very long series of days, and since large hourly peaks were not frequent. This may be illustrated by the example of *Haemagogus equinus*. The activity cycle shown for this species in Table 5 is based on 1,944 hours of collecting during 258 days, when the maximum number taken during any one hour was only eight specimens.

The hourly distribution of the 19 species of which more than 100 specimens were taken during the two-year period of the survey are shown in Table 5. In arriving at these figures we eliminated from consideration any days on which no specimens of a particular species were taken, and for the remaining days determined the number of specimens present per ten man-hours of collecting. Hours during which for some reason, such as heavy rain, the collectors were not at their stations, were also eliminated in arriving at the catch per ten man-hours. For ready comparison of the hourly occurrence of the different species, the attack rates per ten man-hours were converted into percentages of the catch made during each of the six one-hour periods between 9 A.M. and 3 P.M. To give some idea of the relative reliability of the results the size of the sample is shown for each species. Where 90 per cent or more of the catch of a particular species was taken at either canopy or ground level the averages were derived from the

TABLE 5

Comparison of the hourly attack rates of forest mosquitoes given as the percentages of the total number attacking between 9 A.M. and 3 P.M.

Species	0900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	Where taken	[1]Number taken
Haemagogus equinus	11.7	15.7	17.3	18.4	16.1	20.9	Canopy	1,126
Haemagogus lucifer	14.0	14.0	17.3	18.3	16.4	20.0	Canopy	970
Haemagogus spegazzinii falco	12.3	16.2	17.9	18.8	16.5	18.3	Canopy	1,136
Aedes leucocelaenus clarki	14.6	18.3	17.8	15.5	14.9	18.9	Canopy	810
Aedes leucotaeniatus	22.7	21.6	14.8	15.9	11.4	13.6	Can. & Gr.	297
Aedes sexlineatus	27.4	16.4	17.8	17.3	8.2	12.8	Canopy	129
Aedes angustivittatus	28.1	13.4	14.1	12.4	14.1	17.9	Ground	444
Aedes serratus group	21.6	14.3	16.5	18.7	12.7	16.2	Ground	1,735
Psorophora ferox	11.4	13.6	15.9	18.1	20.0	21.0	Can. & Gr.	2,451
Psorophora lutzii	11.0	13.8	22.0	19.3	16.3	17.6	Can. & Gr.	917
Trichoprosopon digitatum	13.0	10.8	15.7	20.0	23.1	17.3	Ground	145
Trichoprosopon magnus	19.9	17.6	15.9	14.5	15.5	16.6	Can. & Gr.	668
Trichoprosopon espini	35.3	17.4	13.7	12.6	6.3	14.7	Can. & Gr.	172
Trichoprosopon leucopus	20.0	17.6	18.0	14.4	14.8	15.2	Ground	438
Limatus asulleptus	10.2	22.0	21.0	18.5	14.6	13.7	Ground	478
Sabethes cyaneus	11.3	13.6	22.6	16.9	15.8	19.8	Can. & Gr.	321
Sabethes tarsopus	12.8	16.8	15.7	15.7	12.8	26.2	Canopy	229
Sabethes chloropterus	14.9	16.1	17.6	16.7	15.8	18.9	Canopy	3,545
Anopheles neivai	23.5	15.7	14.7	18.1	9.3	18.6	Can. & Gr.	154

[1] Hourly attack rates have been calculated only for those species of which more than 100 specimens were taken during the two year period of the study.

catches in that one situation. Where the vertical stratification was less extreme, *i.e.*, species with less than 90 per cent of their numbers at either canopy or ground, the data from the two environments were combined. Which set of data was used for each species is indicated in the table.

Examination of the results summarized in Table 5 first reveals that the variations by hours in the capture rates for most species are not of very great magnitude. As the collections were distributed among six one-hour periods, the proportion taken in any one-hour period, if the mosquitoes were uniformly active between 9 A.M. and 3 P.M., would be one-sixth of 100 per cent or 16.7 per cent. In the case of only one species, *Trichoprosopon espini*, was this theoretical average more than doubled; while but two species, *T. espini* and *A. neivai* had hour periods during which less than half the theoretical average was taken. While the differences in magnitude are slight, three patterns of activity may be detected.

1. Species with the least activity during the first hour (9–10 A.M.) and the most activity during the last hour (2–3 P.M.).

Six species met these conditions as follows: *Haemagogus equinus* and *lucifer*, *Aedes leucocelaenus clarki*, *Psorophora ferox*, *Sabethes tarsopus*, and *chloropterus*. *H. spegazzinii falco* also follows this pattern although the 12–1 P.M. collection was slightly higher (0.5 per cent) than the 2–3 P.M. collection. All of these species also showed a slight decline in the 1–2 P.M. collection with the exception of *P. ferox*. The calculations for all these species except *P. ferox* are based on the

canopy collections only, as they were species of which more than 90 per cent were taken in the canopy.

2. Species with the most activity during the first hour (9–10 A.M.).

This category included eight species as follows: *Aedes leucotaeniatus, sexlineatus, angustivittatus* and the *serratus* group, *Trichoprosopon magnus, espini* and *leucopus*, and *Anopheles neivai*. All but the following three showed some decline during the 1–2 P.M. period as in the case of the first group: *A. angustivittatus, T. magnus* and *leucopus*. With the exception of *A. sexlineatus*, a canopy species, the data used were either from the ground collections or combined ground and canopy collections. *A. neivai*, not exclusively a diurnal species, was most abundant in the hours closest to darkness, 9–10 A.M. and 2–3 P.M., though it also had a secondary peak near midday, noon to 1 P.M.

3. Species most active at an intermediate hour.

The remaining four species had their greatest activity at some intermediate hour. The peak hour for *Limatus asulleptus* was 10–11 A.M., for *Psorophora lutzii* and *Sabethes cyaneus* 11–12 A.M., and for *Trichoprosopon digitatum* 1–2 P.M. With the exception of *T. digitatum* the least active hour for these species was the first hour of collecting, 9–10 A.M., as in the first group. The data for these species were drawn from either the ground or the ground and canopy collections combined.

Whether the slight differences which appear in the daily activity cycles are significant, we think must remain in doubt. Comparable data taken in other parts of Panama reveal somewhat different cycles, though this may be due to differences in forest type and weather. Further analyses of these data by other means may be made at a later date. For the present, one can only generalize that a substantial portion of the biting by the diurnal mosquitoes in the Almirante rain-forest occurred during each of the hours between 9 A.M. and 3 P.M.

Regularity of attack rates

The effectiveness of a mosquito species as a vector of yellow fever is a function of the extrinsic incubation period of the virus at the temperatures to which the mosquito is subjected in nature, and also the relation of this incubation period to the natural life span of the mosquito. But also of importance is the regularity of contact made by the species with susceptible hosts over long periods of time. A species which multiplies virus slowly, but which is long-lived and consistently and frequently bites susceptible hosts throughout the year may be of as much importance in maintaining the transmission cycle as a species which mutiplies virus more rapidly and with greater regularity but which is in less frequent contact with susceptible hosts during some part of the year. The annual abundance cycles presented in the next section throw light on this point, but it is also of interest to examine certain relationships expressed in Table 6. We have selected for illustration in this table the data obtained for the three species of *Haemagogus, Aedes leucocelaenus clarki* and *Sabethes chloropterus*, because of their known or suspected roles in the transmission of sylvan yellow fever, and for *Psorophora*

TABLE 6

Various measures of biting activity

	Haemagogus equinus		*Haemagogus lucifer*		*Haemagogus spegazzinii falco*		*Aedes leucocelaenus clarki*		*Sabethes chloropterus*		*Psorophora ferox*	
	Canopy		Canopy		Canopy		Canopy		Canopy		Ground	
Total no. of mosquitoes taken	1,126		970		1,136		810		3,545		2,185	
No. per 10 man-hrs. of collecting	5.8		5.0		5.8		4.2		18.2		11.2	
Proportion of each canopy sp. taken (%)	15		13		15		11		47		—	
Total no. of days of collecting	338		338		338		338		338		342	
Total no. of hrs. of collecting	1,944		1,944		1,944		1,944		1,944		1,957	
Max. no. of mosquitoes per 1 hr. period	8		9		12		6		21		68	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%
Regularity of attack												
Days during which mosquitoes were taken	258	76	242	72	226	67	234	69	324	96	133	39
Hrs. during which mosquitoes were taken	685	35	602	31	591	30	558	29	1,345	69	427	22
Grouping of mosquitoes attacking												
Mosquitoes taken per hr.												
1	434	39	388	40	355	31	397	49	501	14	173	8
2	290	26	262	27	236	21	208	26	706	20	120	6
3	174	15	132	14	144	13	111	14	540	15	120	6
4	104	9	92	9	104	9	44	5	452	13	116	5
5	70	6	50	5	80	7	20	2	400	11	110	5
6–10	54	5	46	5	183	16	30	4	718	20	458	21
over 10	0	0	0	0	34	3	0	0	228	6	1,088	50

ferox to illustrate certain differences in activity pattern. *H. spegazzinii falco* and *A. leucocelaenus clarki* are well established vectors (Whitman, 1951). It has also recently been established in the laboratory (Galindo *et al.*, 1957) that Middle American *H. equinus* and *S. chloropterus* are capable of transmitting yellow fever, and virus has been recovered from these species in nature (Johnson and Farnsworth, 1956). As it appears increasingly likely that all *Haemagogus* are capable of transmission, we include also *H. lucifer*.

We see in Table 6 (lines 1 to 3) the relative abundance of these species. *S. chloropterus* was approximately three to four times as abundant as the other four canopy species. It is of interest to compare the densities of the populations of two of these species with those reported from sylvan yellow fever areas in Colombia (Bates, 1944 and 1945) and Brazil (Causey and Dos Santos, 1949). (Bates' *Haemagogus capricornii* is now known as *spegazzinii* and his *Sabethoides imperfectus* is the same as our *chloropterus*.) The average density over a year period found by Bates (1945) for *spegazzinii* was 58.8 per ten man hours, for *chloropterus*, 7.8 per ten man hours. Comparison of these figures with those in Table 6 (line 2) reveals that the relative densities of these two mosquitoes is reversed, *spegazzinii* being more abundant in Colombia and *chloropterus* more abundant at Almirante. The density of *spegazzinii* in Colombia was about ten times that at Almirante and three and a half times that of all three species of

Haemagogus combined at Almirante. Bates (1944) records attack rates for *spegazzinii* in the canopy as high as 140 per man hour while Causey and Dos Santos (1949) took as many as 39 *spegazzinii* per man hour. At Almirante the greatest number of *spegazzinii* taken in any one hour was 12 (Table 6, line 6), and this number was attained during only one hour of the 1,944 hours during which collections were made in the canopy. The maximum hour catch for the other two *Haemagogus* at Almirante was eight for *equinus* and nine for *lucifer*. Of all the *Haemagogus* taken at Almirante only 34 were gotten at rates in excess of ten per man hour (Table 6, line 15).

The different balance between the numbers of *Haemagogus* and *Sabethes* in Colombia and Panama suggests that there may be a difference in the relative role of the two kinds of mosquitoes in the transmission of sylvan yellow fever in the two areas. It is of interest therefore to examine further certain numerical relationships between the populations of *Sabethes chloropterus* and the *Haemagogus* species at Almirante. As is pointed out above, the regularity of biting, day by day, may be of as much importance for disease transmission as the actual numbers present. In Table 6 we have also shown the proportions of the total days and hours of collecting during which the various species attacked.

We see that in terms of days (Table 6, lines 4 and 7) all the canopy species were present at least two-thirds of the time; *spegazzinii falco* was least frequent (67 per cent of the days) and *chloropterus* most frequent (96 per cent of the days). The demonstration of the ability of *chloropterus* to transmit yellow fever, together with the above evidence of the regularity with which it attacks in the canopy, cannot but impress one with the possibility that this species may play a role in the maintenance of yellow fever in arboreal primates.

The relative lack of regularity in the appearance of *spegazzinii falco* is also shown by comparison of the data for this species with that for *equinus* and *leucocelaenus clarki* (Table 6, lines 1 and 7). *Leucocelaenus clarki* (810 specimens) was substantially less abundant than *spegazzinii falco* (1,136 specimens), but appeared on a slightly greater number of days: *clarki*—234 days; *falco*—226 days. The numbers of *spegazzinii falco* (1,136) and *equinus* (1,126) were almost the same; but the former was taken attacking during fewer days: *falco*—226 days; *equinus*—258 days. As the *spegazzinii falco* were taken during fewer days, it follows that it attacked in larger numbers on the days when it was present. This may be confirmed by examining the figures for the distribution of the catches by attack rate per hour shown in Table 6, lines 9 to 15. Thus, if regularity of attack is significant in assessing the transmission role, as it would seem to be, then *spegazzinii falco* would appear to be of lesser importance than the other species, other factors such as life span, rate of virus multiplication, *etc.*, being equal.

A further measure of the regularity of attack is that gotten by determining the proportion of the total number of collecting hours during which each of the species was taken (Table 6, lines 5 and 8). In these terms the difference between *chloropterus* and the other species is proportionately more pronounced. The proportion of the hours during which *chloropterus* attacked (69 per cent) was about twice that of the other canopy species (29 to 35 per cent).

TABLE 7

Comparison of the annual cycles of diurnal forest mosquitoes during a two-year period, by months, per ten man-hours of collecting

Species	[1]Year period	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	[2]Where taken	Number taken
Haemagogus equinus	1951–52	6.1	9.6	7.7	4.7	2.4	2.4	11.2	3.1	8.5	4.3	1.3	6.0	Canopy	554
	1952–53	5.1	13.5	8.8	4.8	1.5	6.5	5.3	2.5	13.9	4.2	3.9	0.6	Canopy	572
Haemagogus lucifer	1951–52	15.0	5.7	11.3	6.6	5.0	4.0	4.7	2.8	3.8	7.5	3.4	2.5	Canopy	571
	1952–53	1.8	6.7	6.4	6.4	2.2	3.2	3.1	4.0	6.5	3.0	5.0	0.9	Canopy	399
Haemagogus spegazzinii falco	1951–52	10.0	13.5	23.1	11.6	6.6	1.5	7.0	7.7	3.4	6.2	2.3	1.0	Canopy	792
	1952–53	2.7	9.6	7.5	4.4	3.7	1.9	2.2	3.8	4.4	0.5	0.0	0.6	Canopy	344
Aedes leucocelaenus clarki	1951–52	1.9	1.9	3.5	4.4	2.3	2.9	5.0	4.4	3.3	3.5	1.1	1.2	Canopy	297
	1952–53	1.1	2.9	6.9	5.8	4.6	5.1	9.3	10.3	8.9	7.2	4.2	1.3	Canopy	513
Aedes leucotaeniatus	1951–52	0.6	1.0	1.0	0.9	0.6	0.1	1.5	0.6	0.7	0.4	0.2	0.7	Can. & Gr.	138
	1952–53	0.8	3.0	1.9	0.5	0.3	0.3	0.2	0.3	1.0	0.3	0.5	0.1	Can. & Gr.	159
Aedes sexlineatus	1951–52	0.6	1.4	1.8	0.5	0.2	0.3	0.0	0.0	0.5	0.3	0.1	0.0	Canopy	52
	1952–53	0.0	1.8	2.4	0.1	0.9	0.5	0.6	0.6	1.3	0.5	0.3	0.1	Canopy	77
Aedes angustivittatus	1951–52	0.0	0.2	0.9	1.4	0.1	0.0	0.1	1.6	0.6	0.1	0.0	0.1	Ground	47
	1952–53	0.5	0.1	2.4	14.2	7.0	1.6	8.6	9.2	6.1	3.0	0.0	0.0	Ground	397
Aedes serratus group	1951–52	1.1	0.9	5.9	5.4	2.5	0.5	3.0	3.1	1.1	3.5	0.4	0.4	Ground	242
	1952–53	0.5	3.1	22.7	52.5	5.0	0.3	49.7	16.3	21.9	9.9	0.4	0.0	Ground	1,493
Psorophora ferox	1951–52	0.0	1.0	1.1	1.8	0.1	0.1	0.0	0.1	0.7	0.0	0.0	0.0	Can. & Gr.	99
	1952–53	0.6	0.9	44.8	21.9	1.9	1.1	42.5	7.8	7.9	1.4	0.0	0.0	Can. & Gr.	2,352
Psorophora lutzii	1951–52	0.4	0.5	1.8	3.6	0.0	0.0	0.0	0.2	2.5	0.0	0.0	0.3	Can. & Gr.	182
	1952–53	0.1	0.3	2.0	9.5	0.0	0.1	35.6	0.1	0.9	0.1	0.3	0.0	Can. & Gr.	735
Trichoprosopon digitatum	1951–52	0.8	2.1	0.8	0.9	0.2	0.3	1.1	0.5	1.4	1.1	0.2	2.0	Ground	100
	1952–53	1.0	0.7	0.7	0.6	0.4	0.0	0.0	0.5	0.0	0.0	0.1	1.2	Ground	45
Trichoprosopon magnus	1951–52	1.5	1.9	2.0	1.1	0.5	3.6	3.0	3.8	1.5	3.3	2.8	3.8	Can. & Gr.	484
	1952–53	3.6	0.9	1.3	0.5	1.2	0.2	0.5	0.8	0.9	0.4	0.2	0.9	Can. & Gr.	184
Trichoprosopon espini	1951–52	0.0	0.4	0.8	0.1	0.2	0.3	0.7	0.9	0.6	0.8	0.4	0.6	Can. & Gr.	95
	1952–53	0.6	0.5	0.4	1.3	0.7	0.0	0.5	0.1	0.2	0.2	0.0	0.1	Can. & Gr.	77
Trichoprosopon leucopus	1951–52	0.0	0.1	0.0	0.0	0.2	1.9	2.8	2.1	0.8	2.9	6.0	2.1	Ground	157
	1952–53	1.8	3.9	9.9	5.2	3.5	0.3	1.2	3.5	1.3	1.2	0.2	0.4	Ground	281

Limatus asulleptus	1951–52	0.0	1.4	0.1	1.0	2.4	1.2	3.0	1.3	1.8	2.9	2.7	1.7	Ground	165
	1952–53	2.6	9.5	6.3	5.9	3.1	2.1	1.9	2.9	0.6	1.0	0.8	0.5	Ground	313
Sabethes cyaneus	1951–52	0.8	0.5	0.7	0.4	0.6	1.6	1.2	1.8	0.9	0.9	1.4	1.0	Can. & Gr.	201
	1952–53	0.4	0.2	0.1	0.4	0.8	0.5	0.5	0.8	1.2	0.9	1.1	0.9	Can. & Gr.	120
Sabethes tarsopus	1951–52	1.1	1.1	0.8	1.0	1.8	0.8	0.5	1.0	0.4	0.8	0.7	0.7	Canopy	90
	1952–53	1.9	0.9	0.8	1.9	1.5	0.3	1.2	1.3	1.5	2.1	2.4	2.0	Canopy	139
Sabethes chloropterus	1951–52	11.1	22.1	16.3	16.5	27.0	26.6	10.9	16.1	9.8	11.7	10.3	15.0	Canopy	1,722
	1952–53	15.8	17.9	12.6	13.6	24.8	20.3	19.9	32.5	25.7	33.5	18.5	13.6	Canopy	1,823
Anopheles neivai	1951–52	0.4	0.9	2.8	1.2	0.3	0.3	0.6	0.2	0.6	0.3	0.1	0.0	Can. & Gr.	139
	1952–53	0.1	0.2	<0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.1	0.1	Can. & Gr.	15

[1] As the work was begun in May 1951 the "year periods" extend from May 1951 to April 1952 and from May 1952 to April 1953.

[2] Annual abundance cycles have been calculated only for those species of which more than 100 specimens were taken during the two year period of the study.

Finally, if we examine the data showing the numbers of each species attacking at various rates per hour of collection (Table 6, lines 10 to 16) we find another characteristic of *chloropterus*. That is, aside from the fact that *chloropterus* was more regularly present, as shown above, it was more often present in groups rather than singly as were the other species. In Table 6, lines 10, 11 and 12, we see that for the three species of *Haemagogus* and *leucocelaenus clarki* the numbers taken at the rate of two or three per hour were less than the numbers taken at the rate of one per hour. But *chloropterus* attacked more frequently at rates of two or three per hour than at rates of one per hour. Many more were also taken at rates in excess of five per hour. Thus we see that the greater abundance of *chloropterus* is expressed both in greater regularity of attack and also greater numbers attacking per hour. It might be thought that this relationship must be expected in the case of a species so much more abundant than the others. But it is possible for a species to be abundant and yet not express this abundance both in terms of regularity of appearance and large grouping of individuals.

We include data for *Psorophora ferox* in Table 6 to illustrate this point. This species was about two-thirds as common as *chloropterus* but the maximum catch in a single hour period was more than three times that of *chloropterus* (Table 6, line 6). The clustering of the catch is also shown by the fact that 50 per cent of those taken were in groups in excess of ten per man-hour while only six per cent of the *chloropterus* were taken in such aggregations (Table 6, line 15). The lack of regularity of attack by *P. ferox*, on the other hand, is brought out by the fact that it was taken on only 29 per cent of the days and 22 per cent of the hours of collecting; *chloropterus* being present on 96 per cent of the days and 69 per cent of the hours (Table 6, lines 7 and 8). Although two to two-and-a-half times as abundant as the species of *Haemagogus* and *Aedes leucocelaenus*, *ferox* was taken on substantially fewer days and hours than these species (Table 6, lines 7 and 8).

Annual abundance cycle

An important reason for making the collections at the Almirante stations over a two-year period was that of obtaining a picture of the long-term abundance cycle of the forest mosquitoes in a typical tropical rain-forest which supported the yellow fever transmission cycle. We were interested not only in the relative abundance of the various species from year to year, but also how they might vary from month to month within the period of a year. The gross differences in the numbers of the various species taken during each year period have been shown in Table 2. But for the 19 species of which more than 100 individuals were collected we have calculated the catch per ten man-hours for each month during the two year period. The results are summarized in Table 7. To portray the fluctuations graphically and to facilitate comparisons of species illustrating particular points, the monthly ten man-hour capture rates were converted to percentages of each year's catch taken each month. The annual abundance cycles determined in this way for six selected species are shown in the accompanying circle graphs.

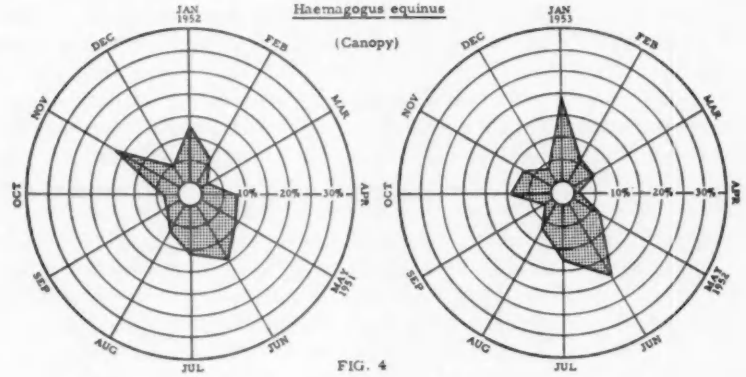


FIG. 4

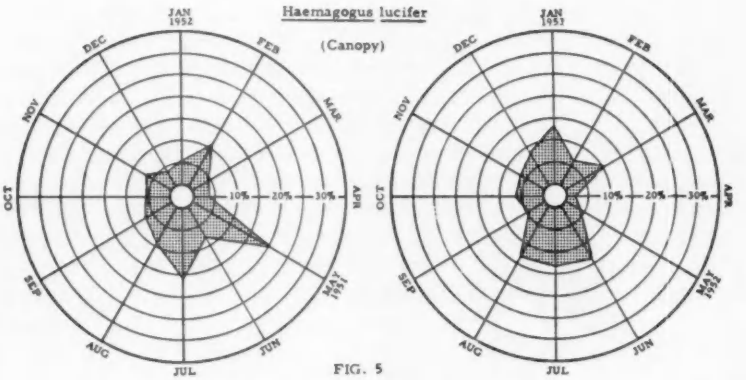


FIG. 5

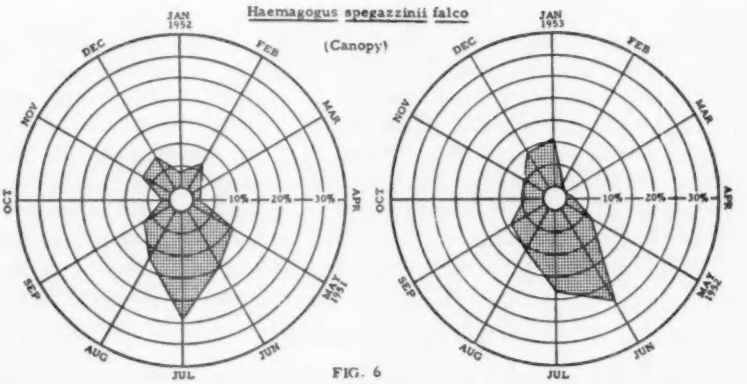


FIG. 6

FIGS. 4, 5, and 6. Annual abundance cycles of three species of *Haemagogus* during a two year period in the tropical rain-forest near Almirante, Panama.

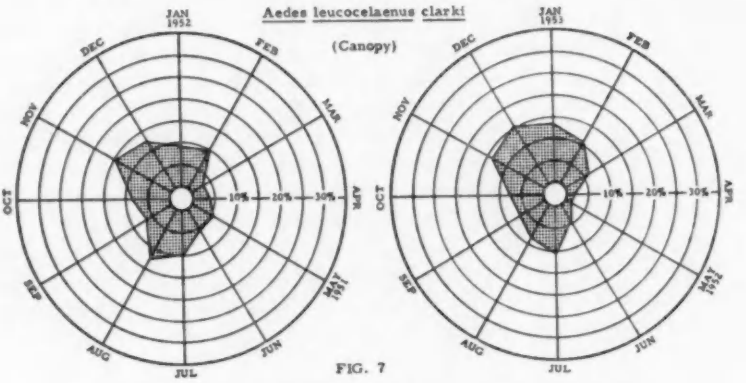


FIG. 7

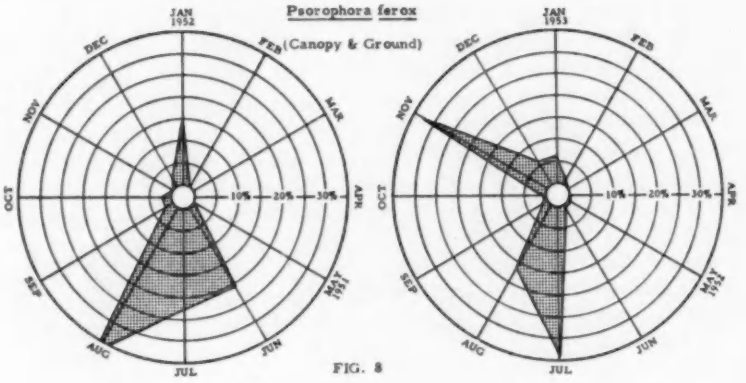


FIG. 8

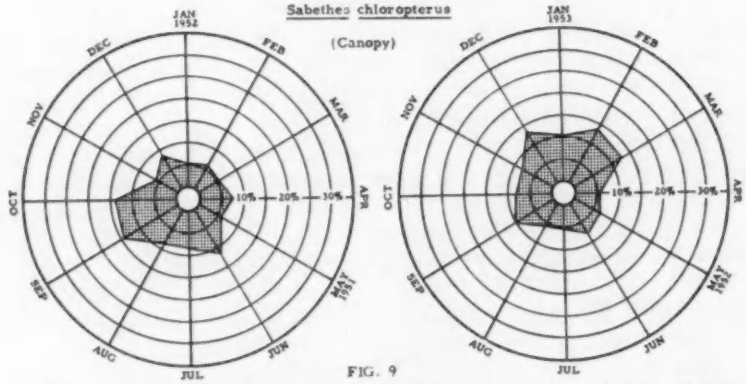


FIG. 9

FIGS. 7, 8, and 9. Annual abundance cycles of three species of mosquitoes during a two year period in the tropical rain-forest near Almirante, Panama.

The irregularity of the details of the annual distribution is apparent on study of Table 7 and the accompanying graphs. In the case of only one species, *Aedes sexlineatus*, was the month of maximum abundance the same (July) during both years. Six other species had their month of maximum abundance one month apart: *Haemagogus lucifer* and *spegazzinii falco*, *Aedes leucocelaenus clarki*, *Psorophora ferox*, *Sabethes cyaneus* and *Anopheles neivai*. The mosquitoes demonstrating the greatest variation in the annual abundance cycle were the ground-pool breeding species of *Aedes* and the *Psorophora*. This is graphically illustrated for one of these species, *P. ferox*, in Figure 8. In such species there are a number of months in which there is no catch at all and there are peaks of abundance when more than 30 per cent of the year's catch is taken in a single month.

The three species of *Haemagogus* (Figures 4, 5 and 6) have pronounced peaks, but these are much less extreme than those of the ground-pool breeding species. The largest peaks for any particular month reach only 20 per cent of the year's catch, and there are no months during which these species are wholly absent. The cycle is even more uniform in the case of *leucocelaenus clarki* (Figure 7) with no peak exceeding 15 per cent. Most uniform in distribution throughout the two-year period was *chloropterus* (Figure 9). A substantial portion of the catch was taken during all months. There was no month in which less than five per cent or more than 15 per cent of the annual catch appeared.

The relative uniformity of the month-by-month catch of the tree-hole-breeding arboreal mosquitoes in this tropical rain-forest must be an important factor in maintaining the chain of yellow fever transmission. In forests which experience a pronounced dry season, as do those of the Pacific versant of Panama, the annual abundance cycles are very different. The contrasting of the cycles in the two types of forest has been made the subject of a separate paper (Trapido and Galindo, 1957b).

SUMMARY

An analysis has been made of the composition of the sylvan mosquito fauna of a well developed virgin tropical rain-forest in which yellow fever appeared in 1951. The results are based on two years of collecting mosquitoes attacking human baits on the ground and in the forest canopy, during the daylight and evening hours. The circumstances under which yellow fever occurred are given, and the climate and microclimate of the rain-forest summarized. It is shown that in true rain-forest the vertical stratification of the sylvan mosquito fauna is much more pronounced than in open deciduous forest. The daily activity cycles of 19 species are illustrated, and an analysis made of the regularity of attack in the case of five species known or suspected as sylvan yellow fever vectors. In addition the annual abundance cycles of 19 species are given.

ACKNOWLEDGEMENTS

This study was made possible by the generous financial support of the Medical Department of the United Fruit Company, arranged by the Medical Director of the Company, Dr. Edward I. Salisbury. We are grateful not only to Dr. Salisbury but also to the local officials of the Chiriqui Land Company in Panama

who lent every assistance in carrying on the work: Dr. Gustav Engler, Medical Superintendent of the Almirante Hospital, Mr. V. T. Mais, Agent, and Mr. G. D. Munch, Assistant Manager. Sr. Audiberto Quiñonez and Sr. Wilfred Hils, technicians in charge of the field work, deserve commendation for the exemplary manner in which they accomplished the day to day routine in the isolated and uninhabited forest where this study was made.

REFERENCES

ALLEE, W. C., 1926. Measurement of environmental factors in the tropical rain-forest of Panama, *Ecology* **7:** 273–302.

BATES, M., 1944. Observations on the distribution of diurnal mosquitoes in a tropical forest, *Ecology* **25:** 159–170.

BATES, M., 1945. Observations on climate and seasonal distribution of mosquitoes in eastern Columbia, *J. Animal Ecol.* **14:** 17–25.

BATES, M., 1947. The stratification of mosquitoes in cages, *Ecology* **28:** 80–81.

CAUSEY, O. R., AND DOS SANTOS, G. V., 1949. Diurnal mosquitoes in an area of small residual forests in Brazil, *Ann. Ent. Soc. Amer.* **42:** 471–482.

ELTON, N. W., 1952. Yellow fever in Panama: historical and contemporary, *Am. J. Trop. Med. & Hyg.* **1:** 436–456.

FAIRCHILD, G. B., 1953. Arboreal Tabanidae in Panama, *Proc Ent. Soc. Wash.* **55:** 239–243.

FAIRCHILD, G. B., AND HERTIG, M., 1952. Notes on the *Phlebotomus* of Panama. IX. Descriptions of seven new species, *Ann. Ent. Soc. Amer.* **45:** 505–528.

GALINDO, P., CARPENTER, S. J., AND TRAPIDO, H., 1951. Description of two new species of *Wyeomyia* and the male of *Sabethes tarsopus* Dyar and Knab (Diptera, Culicidae), *Proc. Ent. Soc. Wash.* **53:** 86–96.

GALINDO, P., RODANICHE, E. C. DE, AND TRAPIDO, H., 1956. Experimental transmission of yellow fever by Central American species of *Haemagogus* and *Sabethes chloropterus*, *Amer. J. Trop. Med. & Hyg.* **5:** In press.

GALINDO, P., TRAPIDO, H., AND CARPENTER, S. J., 1950. Observations on diurnal forest mosquitoes in relation to sylvan yellow fever in Panama. *Amer. J. Trop Med.* **30:** 533–574.

HADDOW, A. J., 1954. Studies of the biting-habits of African mosquitoes. An appraisal of methods employed, with special reference to the twenty-four-hour catch, *Bull. Ent. Res.* **45:** 199–242.

JOHNSON, C. M., AND FARNSWORTH, S. F., 1956. Results of recent studies of yellow fever in Middle America, *Bol. of. san. panamer.* **40:** In press.

KOMP, W. H. W., AND KUMM, H. W., 1938. A new species of *Haemagogus*, *mesodentatus*, from Costa Rica, and a description of the larva of *Haemagogus anastasionis* Dyar (Diptera, Culicidae), *Proc. Ent. Soc. Wash.* **40:** 253–259.

KUMM, H. W., AND ZUÑIGA, H., 1942. The mosquitoes of El Salvador. *Amer. J. Trop. Med.* **22:** 399–415.

LANE, J., 1953. *Neotropical Culicidae*. Univ. Saõ Paulo, Brazil. pp. 1–1112.

TRAPIDO, H., AND GALINDO, P., 1955. The investigation of a sylvan yellow fever epizootic on the north coast of Honduras, 1954. *Amer. J. Trop. Med. & Hyg.* **4:** 665–674.

TRAPIDO, H., GALINDO, P., AND CARPENTER, S. J., 1955. A survey of forest mosquitoes in relation to sylvan yellow fever in the Panama Isthmian area, *Amer. J. Trop. Med. & Hyg.* **4:** 525–542.

TREJOS, A., AND ROMERO, A., 1954. Clinica y laboratorio de la fiebre amarilla en Costa Rica, *Rev. biol. trop. (Costa Rica)* **2:** 113–168.

TREWARTHA, G. T., 1954. *An Introduction to Climate*. McGraw-Hill Book Co., New York. pp. i–vii, 1–402.

WHITMAN, L., 1951. The arthropod vectors of yellow fever. In *Yellow Fever*, G. K. Strode, ed., McGraw-Hill Book Co., New York. pp. 229–298.

FOREST MOSQUITOES ASSOCIATED WITH SYLVAN YELLOW FEVER IN NICARAGUA

PEDRO GALINDO AND HAROLD TRAPIDO[1]

Gorgas Memorial Laboratory, Panama, R. P.

During 1952 and 1953 eleven human yellow fever fatalities from Nicaragua were reported in the Weekly Epidemiological Reports of the Pan American Sanitary Bureau (Trapido and Galindo, 1956 a). During these two years there were also numerous reports of monkey fatalities received by the Nicaraguan Ministry of Public Health, following similar reports during 1951 in Costa Rica. A number of the forest primate deaths in Costa Rica were shown to have been due to yellow fever (Vargas-Mendez and Elton, 1953). Dr. Jorge Boshell, yellow fever consultant to the Pan American Sanitary Bureau, investigated the Nicaraguan reports and was able to obtain livers from recently dead monkeys in the Rivas Isthmus area in 1952. These gave histopathological evidence of having been due to yellow fever. In 1953 monkeys were reported to have been dying on the Pacific slope, south and southwest of Managua, but tissues from freshly dead animals in which the lesions of yellow fever could be demonstrated were not obtained. While these fatalities may well have been due to yellow fever, definite proof of this is lacking. Previous to these events yellow fever in its sylvan form had not been recognized in Nicaragua.

Because of the appearance of sylvan yellow fever in Nicaragua it seemed desirable to make a survey of the forest mosquito fauna which had not been studied before. In 1953 arrangements were made to collect forest mosquitoes during the rainy season near Villa Somoza in Chontales Province, east of Lake Nicaragua, where both human and monkey fatalities had occurred the previous year. The area selected was along the Rama Road 14 to 32 kilometers east of Villa Somoza where the plateau east of Lake Nicaragua slopes down to the Caribbean coastal plain in the basin of the Rio Mico. As this slope is exposed to the northeasterly trade winds it is an area of relatively high rainfall, and the heavy broad-leaved forest has the aspect of tropical rain-forest. Five sites at elevations between 700 and 1,350 feet were selected as collecting stations.

The collecting method used was similar to that previously employed in Panama (Trapido *et al.*, 1955). That is, a pair of men were stationed in the forest to make collections of mosquitoes which approached to attack, on the ground and on a platform constructed in the tree canopy. The simultaneous collections were made during the day, usually between 9 A.M. and 3 P.M. one day a week at each tree station. The results of these collections, which were made during August, September and November are shown in Table 1.

To obtain some idea of the forest mosquito fauna in an area where sylvan yellow fever had been active the previous year on the Pacific slope, collections were first made for a short time (from June 1st to 12th), several weeks after the rainy

[1] Present address: The Rockefeller Foundation, 49 West 49th Street, New York, N. Y.

TABLE 1

Diurnal forest mosquito captures near Villa Somoza, Nicaragua, during August, September and November 1953

Number of man hours collecting	468	[1]Per cent captured	
	No. taken	Ground	Canopy
Tribe Culicini			
Haemagogus equinus	85	3.8	96.2
Haemagogus spegazzinii falco	41	7.7	92.7
Haemagogus iridicolor	243	20.6	79.4
Haemagogus mesodentatus	2	0.0	100.0
Total *Haemagogus*	371		
Aedes leucocelaenus clarki	6	0.0	100.0
Aedes terrens	2	51.8	48.2
Aedes sexlineatus	1	100.0	0.0
Aedes angustivittatus	1	100.0	0.0
Aedes serratus group	3	100.0	0.0
Psorophora ferox	402	98.4	1.6
Psorophora lutzii	104	98.3	1.7
Total Culicini	890		
Tribe Sabethini			
Trichoprosopon magnus	487	9.5	90.5
Trichoprosopon espini	3	69.2	30.8
Trichoprosopon leucopus	1	100.0	0.0
Wyeomyia arborea	1	0.0	100.0
Wyeomyia (*Dendromyia*) sp.	23	54.1	45.9
Wyeomyia sp.	655	66.3	33.7
Limatus asulleptus	1	0.0	100.0
Sabethes cyaneus	7	0.0	100.0
Sabethes tarsopus	8	0.0	100.0
Sabethes chloropterus	195	5.5	94.5
Total Sabethini	1381		
Tribe Anophelini			
Anopheles eiseni	2	51.8	48.2
Anopheles fausti	4	0.0	100.0
Anopheles neivai	7	0.0	100.0
Chagasia bathanus	12	8.2	91.8
Total Anophelini	25		
Total all species	2296		

[1] Percentages corrected for number of man hours of collecting on the ground and in the canopy.

season had begun, at Valle Menier in Rivas Province, on the isthmus between Lake Nicaragua and the Pacific Ocean. This was an area where there had been heavy mortality of howling monkeys in 1952. During September and October of 1953, because of current reports of monkey mortality southwest of Managua on the Pacific slope of the continental divide, the work at Villa Somoza was interrupted to make collections at Guapinolar in Managua Province. The collections

TABLE 2

Abundance of certain diurnal mosquitoes at Pacific side localities in Nicaragua

Locality	Valle Menier			Guapinolar		
Inclusive collecting dates	June 1–12, 1953			Oct. 23–29, 1953		
No. of hours of collecting	105			84		
	Total no. taken	Per cent		Total no. taken	Per cent	
		Ground	Canopy		Ground	Canopy
Haemagogus equinus	2729	57	43	1609	38	62
Haemagogus anastasionis	25	56	44	42	69	31
Sabethes chloropterus	145	79	21	286	19	81

were carried on there somewhat irregularly at first, but we have seven full days of data gotten between the 23rd and the 29th of October when routine ground and canopy catches were made. For these two stations we had summarized in Table 2 only the captures of species known or suspected as sylvan yellow fever vectors. The rainfall and vegetation cover of these Pacific slope stations differ from the conditions at Villa Somoza. As in Panama, the Pacific side had a severe dry season, approximately four months long, and the natural vegetation is in consequence the tropical deciduous forest type. That is, the canopy on the Pacific side is lower and the woods much more open than those near Villa Somoza.

RESULTS

Certain features of the population dynamics of the arboreal forest mosquito fauna were found to differ substantially from those previously worked out in Panama. In analyzing the Nicaragua data we have come to realize that a rational explanation of the findings can best be made by considering each of the species of interest in terms of its "center of origin" or "center of dispersal." The biological principle involved is that recently evolved species (we believe the entire genus *Haemagogus* to be of recent origin) are in best adjustment with the environment in the region where they arose. An important way in which this is expressed is that the center of origin supports maximum population densities of the particular species. It will be seen below how this concept, in combination with the principle that each species has an optimum ecological niche, explains the Nicaragua findings, and incidentally makes clearer the previous findings in other parts of Middle America.

At the stations near Villa Somoza four species of *Haemagogus* were taken. Most abundant was *Haemagogus iridicolor*. It was about three times as common as *Haemagogus equinus*, which in turn was about twice as prevalent as *Haemagogus spegazzinii falco*. The fourth species, *Haemagogus m. mesodentatus*, was rare, only two specimens being taken during the three months of collecting. The composition of the *Haemagogus* catch at the Pacific side localities, Valle Menier and Guapinolar, was very different. *H. equinus* was the one species present in both areas, and was far more abundant at the Pacific side localities than at Villa

Somoza. The second species on the Pacific slope was *Haemagogus anastasionis*. (We have also taken a few *H. iridicolor* on the Pacific versant of Nicaragua, on incidental collecting trips, but not at the established stations.)

The very great difference in the densities of *H. equinus* in the two regions is a matter of much interest. We know from our studies in Panama (Trapido and Galindo, 1957 b) that there are substantial fluctuations in the densities of these tree-hole breeding mosquitoes at different times of the year, and it is therefore only with caution that short-term catches at different localities may be compared. But the magnitude of the differences in densities observed in Nicaragua are so great that it is evident that they are significant, particularly since all the collections were made during the rainy season, and since high densities at the Pacific slope stations were recorded both early and late in the rainy season. Based on an average collecting day of six hours, the density of *equinus* at Guapinolar was 196 per ten man-hours of collecting, at Valle Menier 260 per ten man-hours, but at Villa Somoza only 1.8 per ten man-hours. The difference in abundance in the two areas confirms a finding made in Panama; namely, that while *equinus* occurs in a number of different habitats, it reaches its maximum development in tropical deciduous forest and is relatively less common in tropical rain-forest. As mentioned above, the Caribbean side stations near Villa Somoza were located on an eastern facing slope where the vegetation has the aspect of rain-forest, while the Pacific side localities were in an area of much more open deciduous forest. The densities of *equinus* found at the Pacific side stations exceeded by far those found at any of the numerous collecting stations operated in Panama (Trapido *et al.*, 1955). We know deciduous forest to be the optimim habitat of *equinus*, and we also know that since this species ranges from Venezuela and Colombia to southernmost Texas (Galindo and Trapido, in preparation), the Pacific side Nicaraguan stations must be near the center of dispersal. Thus, in terms of both favored ecological niche and center of dispersal, the high *equinus* populations of the Pacific side Nicaraguan deciduous forest are understandable.

The failure of *H. spegazzinii falco* to appear in the Pacific side collections is probably due to two factors. First is the fact that Nicaragua is at the periphery of the range of the species, which, with its typical race *spegazzinii*, extends south to northern Argentine and has the center of its distribution far off in the basin of the Amazon. Second is the fact that the work in Panama has shown that this is a species whose optimum habitat is rain-forest (Trapido and Galindo, 1957 c). It persists in the Caribbean slope rain-forest in small numbers as far north as Honduras, but in the unfavorable deciduous forest environment of the Pacific side it extends no farther into Central America than the southern portion of the Rivas Isthmus, a short distance south of the location of the Valle Menier and Guapinolar stations.

H. m. mesodentatus in our experience is an Atlantic side form with the exception of a break through to the Pacific side in the San Jose valley of Costa Rica. It is associated with rain-forest, but is nowhere common south of Guatemala and Mexico. The finding of only two specimens at Villa Somoza is consistent with this distributional picture.

H. anastasionis, on the other hand, is primarily a species of the Pacific side and is characteristic of scrubby second growth or open deciduous forest. Thus its appearance only at the Valle Menier and Guapinolar stations is understandable.

H. iridicolor is a species of restricted range and it is limited to Nicaragua, Costa Rica and western Panama. Its optimum habitat is the Caribbean side rain-forest. It was the commonest *Haemagogus* not only at the stations near Villa Somoza but also in the collections made in the Rio San Juan basin, about 120 miles to the south near San Gerardo, Alajuela Province, Costa Rica, where yellow fever appeared in 1951 (Galindo and Trapido, 1955). The fact that it was the most abundant species at both Villa Somoza and San Gerardo may be explained partly in ecological and partly in distributional terms. It was more abundant than *equinus* at these two localities because the optimum habitat of *equinus* is deciduous forest, while *iridicolor* is better adjusted to rain-forest. It outnumbered *spegazzinii falco* and *m. mesodentatus*, which like it are species reaching their best development in rain-forest, because the center of dispersal of the former is far to the south in the Amazon basin and the center of dispersal of the latter is to the north in Guatemala or Mexico. The San Gerardo and Villa Somoza localities are in a broad sense the center of distribution of *iridicolor*, and it is therefore presumably the best adjusted *Haemagogus* to the environment in this area.

The one other aedine mosquito of interest as a possible yellow fever vector is *Aedes leucocelaenus clarki*. This appeared at the Villa Somoza stations but was rare there, only six specimens being taken. It is a species with a distribution similar to that of *H. spegazzinii*; that is, it is widespread in South America, Central America being at the periphery of its range. Like *H. spegazzinii falco* its range ends in adjacent Honduras. It is even less common than that species in Nicaragua and Honduras.

Two sabethine mosquitoes are of interest as possible natural yellow fever vectors. We have been particularly interested in assessing the possible role of *Sabethes chloropterus* in Panama because adults are more in evidence in the dry season than are those of the aedines. In Panama it occurs in both rain-forest and deciduous forest, and it is similarly distributed in Nicaragua. At the Villa Somoza stations its numbers were only slightly lower than those of *iridicolor*, the most abundant *Haemagogus*, but it was far outnumbered by *equinus* at the Pacific side stations. The commonest sabethine of those identified to species at Villa Somoza was *Trichoprosopon magnus*. This species is of some interest as it was also the commonest arboreal mosquito in an area where yellow fever appeared in Honduras in 1954 (Trapido and Galindo, 1955).

The remainder of the catch made at Villa Somoza, shown in Table 1, is of interest for comparison with lists previously published for various forest localities in Panama, but as the other mosquitoes appear not to be involved in yellow fever transmission insofar as is known, they are not discussed individually here.

The vertical stratification of the catch between the ground level and the canopy at the Nicaraguan stations is shown in the tables. As had been mentioned above, the Villa Somoza stations were in an area where rain-forest conditions were closely approximated. The four species of *Haemagogus*, *Aedes leucocelaenus clarki*, and

the two sabethines, *Trichoprosopon magnus* and *Sabethes chloropterus* appeared preponderantly there in the canopy. But in the open deciduous forest of the Pacific slope, at Valle Menier and Guapinolar, the catches of *Haemagogus* and *S. chloropterus* were much more evenly distributed between the ground and canopy. This confirms a similar finding in Panama (Trapido and Galindo, 1957 c); *i.e.*, that the degree to which essentially arboreal mosquitoes are confined to the tree tops is determined by the density of the covering canopy: in heavy rain-forest the stratification is extreme; in open deciduous forest substantial numbers of the arboreal species attack at ground level.

DISCUSSION IN RELATION TO YELLOW FEVER

H. spegazzinii falco is an established vector of yellow fever in South America (Whitman, 1951). The numbers taken at the Villa Somoza stations were so small that it is doubtful if the species played any important role in maintaining the disease there.

It has recently been demonstrated that *H. equinus* and *H. m. mesodentatus* of Guatemalan origin are capable of transmitting yellow fever by bite (Galindo *et al.*, 1957) and become infected in nature (Johnson and Farnsworth, 1956). The very high density of *equinus* at the Pacific side stations would seem to point to it as the principal vector there, if the heavy monkey mortality in the area is accepted as having been due to yellow fever. The failure of yellow fever to proceed further on the Pacific side of Nicaragua in the years since 1953 may also be explained in terms of *equinus* as the principal vector. The monkey mortality observed in that part of Nicaragua occurred during the rainy season (July 1952 in the southern part of the Rivas Isthmus, and August, September and October 1953 on the Pacific slopes south of Managua). Studies in Panama (Trapido and Galindo, 1957 b) have shown that *H. equinus*, while abundant during the rainy season, is very rare during the severe dry season in deciduous forest such as that in which the large numbers were taken in Nicaragua; and also that high humidity conditions are necessary for the average life span to exceed the extrinsic incubation period of the virus. While we made no dry season collections on the Pacific slope of Nicaragua we are confident that at that time of year *equinus* would be rare or absent, and that such individuals as might be present would be short lived as adults. If *equinus* were the principal species carrying on transmission during the rainy season, as seems likely, it would be difficult for it to maintain the virus cycle over the dry season, and apparently it did not.

H. m. mesodentatus does not occur on the Pacific side of Nicaragua, and was so rare at the Caribbean side stations that one cannot conceive it to have been an element in maintaining yellow fever, even though it is apparently an effective vector in Guatemala.

H. iridicolor, like its close relative *lucifer* of Panama and Colombia, has not been the subject of experiment. But as all *Haemagogus* thus far tested have been shown to be capable of transmitting yellow fever, it seems reasonable to suppose that under suitable conditions *iridicolor* would be a vector. The certain incrimination of *iridicolor* and *lucifer* as vectors, however, will have to await confirmation

by laboratory experiments. But in the rain-forest of the Rio San Juan basin of Costa Rica, and in the Caribbean slope forests of Nicaragua, a region where *iridicolor* is at the center of its distribution and is the commonest *Haemagogus* present, circumstantial evidence would indicate that it did play some part in maintaining transmission.

The ability of *H. anastasionis* to transmit yellow fever has never been experimentally tested, but since it is a member of the same subgenus (*Stegoconops*) as such efficient vectors as *spegazzinii* and *mesodentatus*, it is probably capable of transmitting. The numbers taken, however, were so small in comparison with *equinus* that it is doubtful whether it was of significance in transmission where it was found at the Pacific slope stations.

Aedes leucocelaenus is an established vector of yellow fever in South America (Whitman, 1951) but its race *clarki* was so rare in the Nicaraguan collections that it could not have been an element of any importance in transmission.

In Panama *Sabethes chloropterus* was found to be the forest canopy species whose adult populations best withstand dry season for reasons associated with certain aspects of its life cycle (Trapido and Galindo, 1957 b). Very recently it has been shown to be capable of transmitting yellow fever by bite (Galindo *et al.*, 1957) and virus has been recovered from it in nature (Johnson and Farnsworth, 1956). The numbers taken at the Nicaraguan stations are perhaps sufficient to suppose it was involved in transmission there. We have no dry season data from Nicaragua to use in estimating its possible role at that difficult time of year.

There is little evidence on which to consider the possibility of *Trichoprosopon magnus* as a vector. It has been mentioned in the literature (Whitman, 1951) that a laboratory transmission was accomplished with the Brazilian *Trichoprosopon frontosus*, but the details of the experiments have not been published. As mentioned, above, we found *magnus* to be the commonest arboreal mosquito following an epizootic of yellow fever on the north coast of Honduras (Trapido and Galindo, 1955) but virus was not recovered from it (Rodaniche, 1956). Until it has been the subject of experimental study we can only note with interest that it was the commonest arboreal species in the epizootic area near Villa Somoza.

SUMMARY

The results are given of forest canopy mosquito surveys at three places in Nicaragua where there was evidence of sylvan yellow fever activity during 1952 and 1953. At these localities five species of *Haemagogus* were taken: *spegazzinii falco*, *iridicolor*, *m. mesodentatus*, *equinus* and *anastasionis*, the last named only on the Pacific slope, and the first three only on the Caribbean slope. *H. iridicolor* is also present on the Pacific slope in small numbers but was not in the collections made at the two fixed stations operated there. Also present and of interest as possible vectors were *Aedes leucocelaenus clarki* and *Trichoprosopon magnus* on the Caribbean side only, and *Sabethes chloropterus* on both the Caribbean and Pacific versants. On epidemiological grounds *H. equinus* appears to have been the vector on the Pacific side and *H. iridicolor* the principal vector on the Caribbean slope. The possible role of *S. chloropterus*, considered an important species in

carrying virus over the dry season in Panama, could not be assessed as the Nicaraguan collections were made only in the rainy season. Also in doubt is the possible part played by *Trichoprosopon magnus*, the commonest arboreal mosquito at the Caribbean side stations, since its ability to transmit has not yet been determined. It is thought that the numbers of three species known to be vectors elsewhere, *H. spegazzinii falco*, *H. m. mesodentatus* and *A. leucocelaenus clarki* were too small for them to have been significantly involved in transmission.

ACKNOWLEDGEMENTS

We are indebted to the Nicaraguan public health officials who expedited our work in Nicaragua in many ways, Dr. Leonardo Somarriba, Minister of Public Health, and Dr. Alejandro Robleto Perez, Chief of the Insect Control Division of the Ministry of Public Health. During much of the time the work was being carried on in Nicaragua our field companion was Dr. Jorge Boshell, consultant on yellow fever to the Pan American Sanitary Bureau. He personally supervised the making of the collections at Guapinolar, and we have profited much from his wide experience with sylvan yellow fever.

REFERENCES

GALINDO, P., RODANICHE, E. C. DE, AND TRAPIDO, H., 1957. Experimental transmission of yellow fever by Central American species of *Haemagogus* and *Sabethes chloropterus*, *Amer. J. Trop. Med. & Hyg.* **6:** In press.

GALINDO, P., AND TRAPIDO, H., 1955. Forest canopy mosquitoes associated with the appearance of sylvan yellow fever in Costa Rica, 1951, *Amer. J. Trop. Med. & Hyg.* **4:** 543–549.

JOHNSON, C. M., AND FARNSWORTH, S. F., 1956. Results of recent studies of yellow fever in Middle America, *Bol. of. san. panamer.* **40:** In press.

RODANICHE, E. C. DE, 1956. Survey of mosquitoes captured in Honduras for yellow fever virus, *Amer. J. Trop. Med. & Hyg.* **5:** 480–482.

TRAPIDO, H., AND GALINDO, P., 1955. The investigation of a sylvan yellow fever epizootic on the the north coast of Honduras, 1954, *Amer. J. Trop. Med. & Hyg.* **4:** 665–674.

TRAPIDO, H., AND GALINDO, P., 1956 a. The epidemiology of yellow fever in Middle America, *Exp. Parasitol.* **5:** 285–323.

TRAPIDO, H., AND GALINDO, P., 1957 b. The population dynamics of the forest canopy mosquito fauna in relation to the epidemiology of sylvan yellow fever in Panama, *Amer. J. Trop. Med. & Hyg.* **6:** In press.

TRAPIDO, H., AND GALINDO, P., 1957 c. Mosquitoes associated with sylvan yellow fever near Almirante, Panama, *Amer. J. Trop. Med. & Hyg.* **6:** In press.

TRAPIDO, H., GALINDO, P., AND CARPENTER, S. J., 1955. A survey of forest mosquitoes in relation to sylvan yellow fever in the Panama Isthmian area, *Amer. J. Trop. Med. & Hyg.* **4:** 525–542.

VARGAS-MENDEZ, O., AND ELTON, N. W., 1953. Naturally acquired yellow fever in wild monkeys of Costa Rica, *Amer. J. Trop. Med. & Hyg.* **2:** 850–863.

WHITMAN, L., 1951. The arthropod vectors of yellow fever. In *Yellow Fever*, G. K. Strode, ed., McGraw-Hill Book Co., New York, pp. 229–298.

THE DISTRIBUTION OF *CULEX P. PIPIENS* AND *C. P. QUINQUEFASCIATUS* IN NORTH AMERICA[1]

A. RALPH BARR

Department of Entomology, University of Kansas, Lawrence, Kansas

In recent years a great deal of work has been done on the relationships between various members of the *Culex pipiens* complex. It is now generally recognized that there are at least two more or less well-defined members, *Culex p. pipiens* and *C. p. quinquefasciatus*. The status of *Culex molestus* has not yet been completely investigated but it would appear that it is a strain or group of strains of *pipiens* s.s., possibly an urban biotype. Most of the pertinent literature on this group of mosquitoes has been collected in a symposium published by the Royal Entomological Society of London (Mattingly *et al.*, 1951). Due to the difficulty of differentiating the various members of the complex, their distributions are only vaguely known. This study is designed to provide more complete information on the distributions of these forms in North America.

METHODS

Three forms in the complex are at present known from North America, *pipiens*, *quinquefasciatus*, and "*molestus*". The identification of the latter rests upon a biological characteristic, autogeny; morphologically, the form is difficult to separate from *pipiens*. The present study was done from a morphological point of view; if specimens of "*molestus*" are included they could not be separated from *pipiens*.

The only reliable character for separating individual *pipiens* and *quinquefasciatus* was described by Sundararaman (1949). This is a ratio of two measurements of various parts of the male genitalia. This measurement has been adopted by a succession of workers (Barr and Kartman, 1951; Mattingly *et al.*, 1951; Knight and Malek, 1951; Dobrotworsky and Drummond, 1952; Knight, 1953a, b, c) and has proved reliable.

Securing specimens. A form letter requesting series of these forms was circulated to culicidologists in North America. This resulted in about 150 lots of material, two of which were not from North America. The contributors of this material made the study possible. Individual specimens were not requested and were usually not studied.

Mounting specimens. Each lot was numbered on receipt and stored in alcohol to preclude insect damage. The insects were cleared for a few minutes in hot 10% KOH, washed in water, neutralized in a dilute acetic acid solution, dehydrated in 95% alcohol, cleared and dissected in creosote, and mounted in

[1] This investigation was supported in part by a research grant (E-156) from the Laboratory of Tropical Diseases of the National Institutes of Health, Public Health Service and in part by the Department of Entomology and Economic Zoology, University of Minnesota and the Department of Entomology, University of Kansas. Contribution No. 930 from the Department of Entomology, University of Kansas.

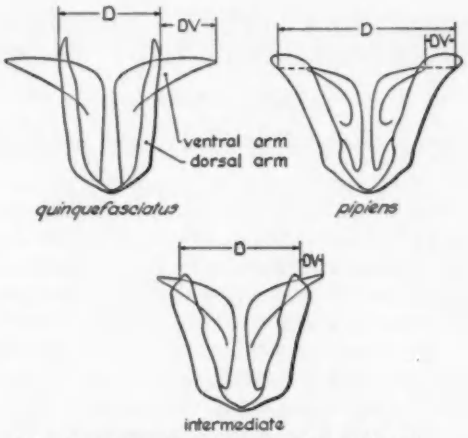


fig 1. Measurements taken of the dorsal and ventral mesosomal arms of the male genitalia of *Culex pipiens*.

balsam. KOH clearing was carried out only long enough to relax the specimens and remove the internal organs; the sclerotized areas were not allowed to become appreciably lighter in color. The genital capsules were handled by lots and were arranged in several rows on a slide; up to 49 individuals were mounted on a slide. This, of course, facilitated measuring the specimens and storing the slides. The capsules were first arranged in rows in a thin sheet of balsam on a slide. Broken bits of cover-slips were arranged around the specimens to support the cover-slip while the preparation was still wet. The slide was allowed to dry for several days and then more balsam and a cover-slip were added. This technique was also used in the study by Barr and Kartman (1951).

Method of measurement. The measurements recorded are as shown in Fig. 1. DV is the extension of the ventral arm laterally of its intersection with the dorsal arm. In specimens with a negative ratio the same measurement was used. Since there was no intersection of the arms, the point where they would have intersected had the ventral arm been longer was used. Both DV measurements were recorded and an average taken. D is the distance between the two intersections of the dorsal and ventral arms. The measurements were made by means of an ocular micrometer at 100 diameters magnification. If too high a magnification is used the exact distances to be measured are more difficult to define. The measurements were estimated to the nearest half of a scale division. Terminalia which were highly distorted were not measured. If the measurements as defined above are compared with those of Sundararaman (1949) and Rozeboom (in Mattingly *et al.*, 1951) differences will be found. The reason for making this change is that the dorsal arms of the phallosome are often not pointed as shown by these authors; this is particularly true of *pipiens* specimens from northern

areas. It is believed that the modification proposed here gives somewhat more objective measurements although the difference is not great.

In the study by Barr and Kartman (1951) negative DV measurements were recorded as being zero. The inclusion of negative values in that study would not have changed the results greatly. It would appear that Sundararaman also did not record negative measurements; Christophers (in Mattingly *et al.*, 1951) first used negative ratios.

From the foregoing it is apparent that these measurements are consistent only within a study and not between different studies. The most important variables are the manner of mounting specimens and the type of measurements taken. It should be pointed out that one of the greatest sources of error is deciding the exact distance to be measured. From these considerations it is apparent that subtle statistical manipulations of the data should be avoided.

RESULTS

After all lots had been mounted and measured, the mean, standard deviation, and standard error of the mean were calculated for each lot. The means of each lot were plotted against latitude (fig. 8). It was found that the average ratio showed a sharp cline in latitudes across the central part of the United States. All lots taken north of this cline were examined and it was found that the ratios of *pipiens* specimens were generally less than 0.2 and the average ratios of lots of *pipiens* were less than 0.1. The same was done with *quinquefasciatus* and it was found that individuals usually had values greater than 0.4 and lots had mean values greater than 0.6. These values were selected as standards for the study. Specimens with a ratio of less than 0.2 were considered to be pipiens, those from 0.2 to 0.4 intermediates, and those with values above 0.4 *quinquefasciatus*. The discussion of individual lots follows.

Canada

Ontario. Ottawa; from D. G. Peterson; a lot of 28 specimens, probably reared; mean $-0.08 \pm .01$[2]; SD[3] 0.05; range -0.15 to $+0.06$; all *pipiens*.

Quebec. St. Anne de Bellevue; October 1944; from J. J. McLintock; a lot of 23 reared males; mean $0.01 \pm .02$; SD 0.08; range -0.10 to $+0.21$; 1 intermediate, 22 *pipiens*. This sample has a single specimen which falls in the intermediate range and morphologically the specimen resembles an intermediate. Since the locality is on the St. Lawrence River it is entirely possible that this is a hybrid. No other intermediates have been found in the northern part of the range of *pipiens*.

United States

Minnesota. St. Paul; Sept. 1953; a lot of 27 males reared by the author; mean $-0.07 \pm .01$; SD 0.06; range -0.21 to $+0.05$. A second sample collected by J. J. Parks contained 6 individuals; Oct. 7, 1953; mean -0.05; range -0.08 to 0.00; *pipiens*.

[2] Standard error of the mean.
[3] Standard deviation.

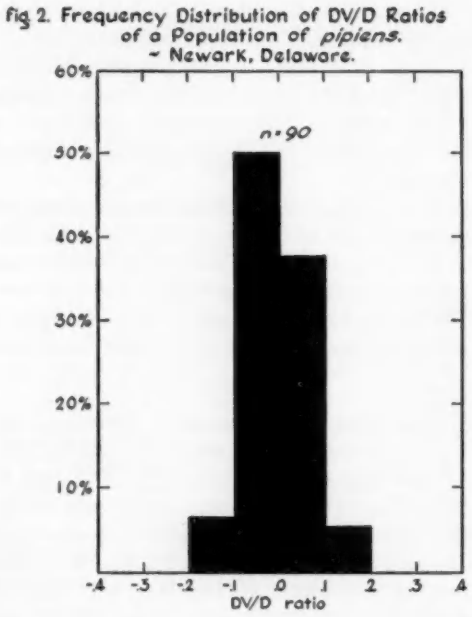


fig 2. Frequency Distribution of DV/D Ratios of a Population of *pipiens*. ~ Newark, Delaware.

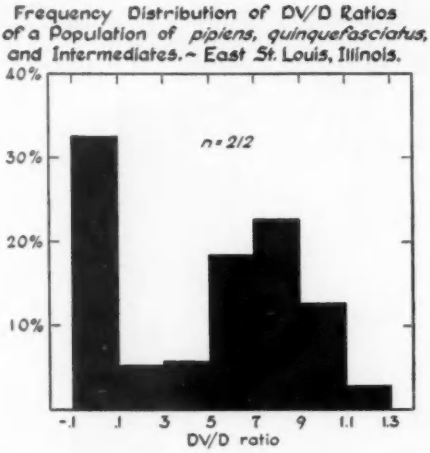
New York. Freeport; Sept. 4, 1953; from G. S. Tulloch; 56 specimens; mean −0.02 ± .01; SD 0.06; range −0.15 to + .11; *pipiens*. Governor's Island; Sept. and Oct. 1953; from G. B. Stansell; 15 specimens; mean 0.00 ± .01; SD 0.04; range −0.05 to +0.06; *pipiens*.

New Jersey. Oxford; Sept. 16, 1953; L. E. Hagmann; 49 reared males; mean −0.07 ± .01; SD .05; range −0.17 to 0.00; *pipiens*.

Delaware. Newark (fig. 2); Aug. 29, 1953; R. F. Darsie; a lot of 90 males, probably reared; mean −0.03 ± .01; SD 0.06; range −0.17 to +0.11; *pipiens*.

Maryland. Several lots from W. E. Bickley. Bainbridge; July 31, 1945; 21 specimens; mean 0.04 ± .01; SD 0.04; range −0.04 to 0.13; *pipiens*. Cambridge; 4 lots taken Aug. 1–6, 1945; 47 specimens; mean 0.04 ± .01; SD 0.05; range −0.05 to +0.15; *pipiens*. Patuxent Naval Air Station, Cedar Point, St. Mary's Co.; Sept. 8 to Oct. 1, 1945; 31 specimens; mean 0.07 ± .03; SD 0.15; range −0.05 to 0.77. This sample contains 30 *pipiens* (highest value 0.19), and 1 *quinquefasciatus*.

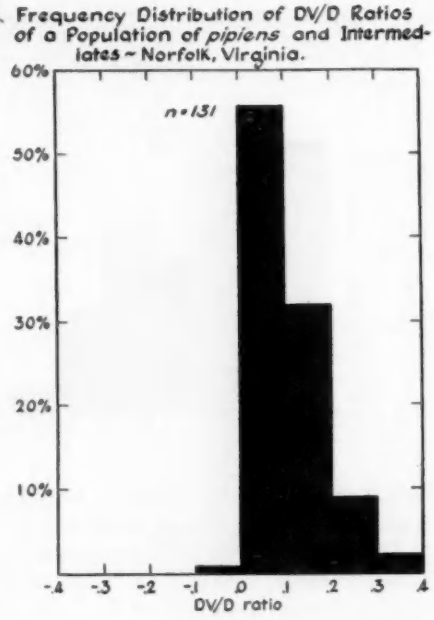
Illinois. Several collections from H. H. Ross. Great Lakes; light trap, 1944 season; 49 specimens; mean −0.02 ± .01; SD 0.06; range −0.11 to +0.12; *pipiens*. Raymond; Oct. 5, 1939; 10 specimens; mean −0.01 ± .01; SD 0.05; range −0.09 to +0.05; *pipiens*. East St. Louis; light trap, Sept. 1, 1943; 15 specimens; mean 0.03 ± .01; SD 0.05; range −0.07 to +0.09; *pipiens*. Another lot from East St. Louis; light trap, 1944 season (fig. 3); 212 specimens; mean 0.48 ± .03; SD 0.37; range −0.07 to +1.25; this collection contains 79 *pipiens*,



8 intermediates, and 125 *quinquefasciatus*. Cahokia; Sept. 16, 1943; taken sweeping; 66 specimens; mean 0.83 ± .03; SD 0.22; range 0.38 to 1.25; 1 intermediate, 65 *quinquefasciatus*. Carmi; Oct. 2, 1941; 12 specimens; mean 0.01 ± .02; SD 0.05; range −0.09 to +0.09; *pipiens*. Illinois Ordnance Plant, Crab Orchard Lake; July 9, 1942; 8 specimens; mean 0.17 ± 0.11; SD 0.32; range −0.02 to +0.92; 7 *pipiens*, 1 *quinquefasciatus*.

Missouri. Kirkwood; from laboratory colony of R. S. Roberts; Aug. 28, 1952; 14 specimens; mean 0.03 ± .01; SD 0.04; range 0.00 to 0.11; *pipiens*. Kirkwood; 3 collections from F. C. Harmston; 121 specimens; mean 0.05 ± .01; SD 0.06; range −0.06 to 0.36; 1 intermediate, 120 *pipiens*. Baden, North St. Louis; May 5, 1952; F. C. Harmston; 6 specimens; mean 0.07 ± .04; SD 0.11; range −0.02 to 0.26; 5 *pipiens*, 1 intermediate. No. St. Louis; June 14, 1952; F. C. Harmston; 10 specimens; mean 0.04 ± .02; SD 0.05; range −0.04 to +0.09; *pipiens*. St. Louis; Oct. 1953; L. E. Ottersbach; 60 specimens; mean 0.85 ± .05; SD 0.37; range −0.05 to 1.83; 5 *pipiens*, 55 *quinquefasciatus*. St. Louis; Aug. 26, 1952; R. S. Roberts; 20 specimens; mean 0.23 ± .06; SD 0.29; range −0.05 to 0.90; 13 *pipiens*, 3 intermediates, 4 *quinquefasciatus*. University City; June 19, 1952; F. C. Harmston; 2 specimens; mean 0.04; *pipiens*. Richmond Heights; June 5, 1952; F. C. Harmston; 22 specimens; mean 0.00 ± .01; SD 0.06; range −0.09 to +0.16; *pipiens*. Joplin; Sept. 5, 1952; R. S. Roberts; 10 specimens; mean 0.14 ± .03; SD 0.09; range 0.02 to 0.24; 7 *pipiens*, 3 intermediates. St. Ann; July 17, 1952; F. C. Harmston; 1 specimen; −0.03; *pipiens*.

Kansas. Seneca; Aug. 13, 1951; R. S. Roberts; 11 specimens; mean 0.02 ± .01; SD 0.05; range −0.05 to +0.13; *pipiens*. Lawrence; July 28, 1951; R. S. Roberts; 15 specimens; mean 0.04 ± .02; SD 0.07; range −0.06 to +0.19; *pipiens*. Lawrence; Aug. 24–28, 1955; reared by H. L. McMillan; 43 specimens; mean 0.04 ± .01; SD 0.08; range −0.05 to 0.36; 2 intermediates, 41 *pipiens*. Frontenac; Aug. 15, 1951; R. S. Roberts; 11 specimens; mean 0.11 ± .02; SD 0.08; range



0.05 to 0.22; 1 intermediate, 10 *pipiens*. Baxter Springs; lab colony from R. S. Roberts; 9 specimens; mean 0.15 ± .03; SD 0.10; range 0.06 to 0.29; 5 *pipiens*, 4 intermediates.

Virginia. Norfolk (fig. 4); a light trap collection from R. E. Dorer; 131 specimens; mean 0.11 ± .01; SD .07; range −0.06 to 0.39; 116 *pipiens*, 15 intermediates.

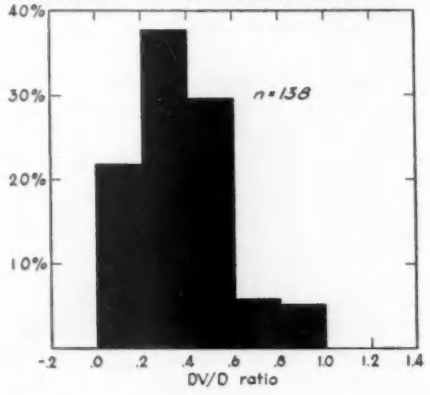
North Carolina. Camp Lejeune; reared by J. D. DeCoursey; 16 specimens; mean 0.92 ± .08; SD 0.32; range 0.58 to 1.50; *quinquefasciatus*.

Georgia. Lakewood, Atlanta; Oct. 1955; H. D. Pratt; 103 specimens; mean 0.91 ± .03; SD 0.29; range 0.49 to 1.44; *quinquefasciatus*. Emory University Field Station, Baker Co.; Sept. 1953; reared by M. H. Goodwin; 28 specimens; mean 0.83 ± .05; SD 0.24; range 0.57 to 1.25; *quinquefasciatus*.

Florida. Orlando; reared by W. C. McDuffie; 103 specimens; mean 0.80 ± .02; SD 0.22 range 0.50 to 1.50; *quinquefasciatus*. Miami; J. H. Heidt; 62 specimens; mean 0.91 ± .04; SD 0.29; range 0.50 to 1.67; *quinquefasciatus*.

Alabama. Several lots from W. E. Snow. Florence; Oct. 12, 1954; 78 specimens; mean 0.79 ± .04; SD 0.31; range 0.11 to 1.61; 2 *pipiens*, 4 intermediates, 72 *quinquefasciatus*. Sheffield; Nov. 18, 1953, reared; 12 specimens; mean 0.77 ± .08; SD 0.30; range 0.55 to 1.23; *quinquefasciatus*. Winfield; 4 specimens; mean 0.64; range 0.61 to 0.71; *quinquefasciatus*. Auburn; Oct. 3, 1953; S. A. Edgar; 25 specimens; mean 0.94 ± .06; SD 0.30; range 0.64 to 1.38; *quinquefasciatus*. Montgomery; laboratory colony of R. W. Chamberlain; 60 specimens; mean 1.12 ± .04; SD 0.28; range 0.80 to 1.67; *quinquefasciatus*.

fig 5 Frequency Distribution of DV/D Ratios of a Population of *pipiens, quinquefasciatus,* and Intermediates.~Sacramento Co., California.



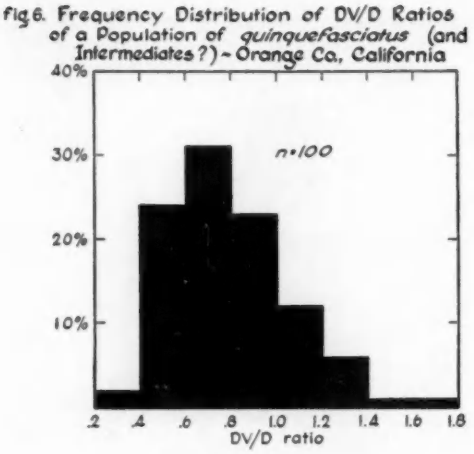
Mississippi. All material from A. G. Peterson; all *quinquefasciatus.* Columbus; Aug. 24, 1944; 44 specimens; mean 0.89 ± .03; SD 0.21; range 0.57 to 1.25. Greenville; Sept. 7 to Nov. 16, 1943 and 1944; 25 specimens; mean 0.75 ± .04; SD 0.22; range 0.50 to 1.13. Tunica; Sept. 7, 1945; one specimen; 0.64.

Arkansas. All material from T. E. McNeel. Gurdon; July 27 to Aug. 8; 14 specimens; mean 0.85 ± .08; SD 0.30; range 0.57 to 1.20; *quinquefasciatus.* Pine Bluff; June 17, 2 specimens; 0.64 and 1.07; *quinquefasciatus.* Russellville; July 14–16; 9 specimens; mean 0.82 ± .12; SD 0.35; range 0.58 to 1.10; *quinquefasciatus.* Bull Shoals; July 1 to Aug. 29; 9 specimens; mean 0.32 ± .09; SD 0.26; range 0.10 to 0.90; 1 *pipiens,* 7 intermediates, 1 *quinquefasciatus.* El Dorado; Sept. 14, 1953; 7 specimens; mean 0.87 ± .18; SD 0.46; range 0.83 to 1.00; *quinquefasciatus.*

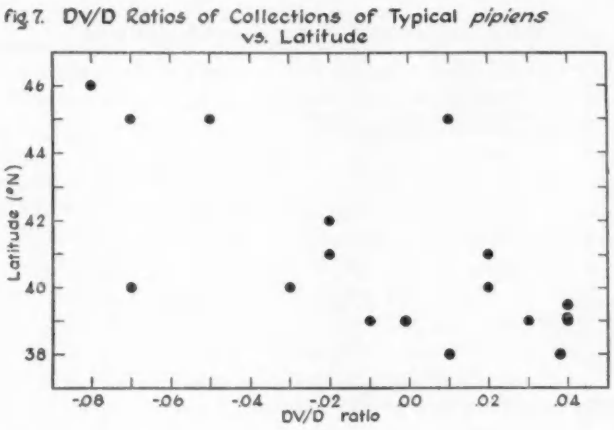
Texas. Plainview; Aug. and Sept. 1953; F. C. Harmston; 17 specimens; mean 0.99 ± .10; SD 0.36; range 0.66 to 1.66; *quinquefasciatus.* Lubbock; Aug. 1953; F. C. Harmston; 42 specimens; mean 1.02 ± .07; SD 0.47; range 0.36 to 2.27; 2 intermediates, 40 *quinquefasciatus.* Beaumont; Aug. 1954; O. P. Breland; 65 specimens; mean 0.87 ± .03; SD 0.22; range 0.52 to 1.36; *quinquefasciatus.* Eagle Pass; Oct. 3, 1953; 2 specimens; 0.70 and 0.80; *quinquefasciatus.* Houston; A. C. Chandler; 76 specimens; mean 0.81 ± .02; SD 0.21; range 0.44 to 1.83; *quinquefasciatus.*

Utah. Salt Lake Co.; 5 collections, various dates, from L. T. Nielsen; 41 specimens; mean 0.08 ± .04; SD 0.23 range −0.10 to 1.50; 40 *pipiens,* 1 *quinquefasciatus.*

California. Sacramento Co. (fig. 5); Aug. 28–Sept. 22, 1947; E. C. Loomis; 138 specimens; mean 0.36 ± .02; SD 0.20; range 0.08 to 0.88; 30 *pipiens,* 52 intermediates, 56 *quinquefasciatus.* San Joaquin Co., E. C. Loomis; 36 specimens; mean 0.61 ± .04; SD 0.21; range 0.19 to 1.00; 1 *pipiens,* 4 intermediates, 31 *quinquefasciatus.* Stockton, San Joaquin Co.; E. C. Loomis; 4 males; mean 0.32;



range 0.13 to 0.58; 1 *pipiens*, 2 intermediates, 1 *quinquefasciatus*. Firebaugh, Fresno Co.; Oct. 7, 1952; E. C. Loomis; 12 specimens; mean 0.71 ± .09; SD 0.31; range 0.43 to 1.13; *quinquefasciatus*. Fresno; Sept. 17, 1953; B. A. Newhouse and G. F. Smith; 69 specimens; mean 0.69 ± .02; SD 0.18; range 0.33 to 1.13; 2 intermediates, 67 *quinquefasciatus*. Fresno, Oct. 16, 1953; R. C. Husbands; 209 specimens; mean 0.76 ± .01; SD 0.19; range 0.38 to 1.38; 1 intermediate, 208 *quinquefasciatus*. Fresno; Oct. 8, 1953; R. C. Husbands; 202 specimens; mean 0.79 ± .02; SD 0.23; range 0.43 to 2.00; *quinquefasciatus*. Selma, Fresno Co.; Nov. 30, 1953; from the Consolidated Mosquito Abatement District; 30 specimens; mean 0.87 ± .05; SD 0.27; range 0.50 to 1.25; *quinquefasciatus*. King's Co.; E. C. Loomis; 4 specimens; mean 0.65; range 0.50 to 0.83; *quinquefasciatus*. Bakersfield; Sept. 16, 1953; B. A. Newhouse and G. F. Smith; 38 specimens; mean 0.74 ± 0.04; SD 0.22; range 0.50 to 1.25; *quinquefasciatus*. Loma Linda, San Bernardino Co.; reared by R. E. Ryckman; 135 specimens; mean 0.87 ± .02; SD 0.25; range 0.36 to 1.67; 1 intermediate, remainder *quinquefasciatus*. San Bernardino, San Bernardino Co.; Apr. 23, 1951; reared by R. E. Ryckman; 57 specimens; mean 0.88 ± .03; range 0.50 to 1.38; *quinquefasciatus*. Cucamonga, San Bernardino Co.; reared by R. E. Ryckman; 11 specimens; mean 0.64 ± .07; SD 0.24; range 0.44 to 0.83; *quinquefasciatus*. Redlands, San Bernardino Co.; Sept. 20, 1951; reared by R. E. Ryckman; 145 specimens; mean 0.90 ± .03; SD 0.29; range 0.44 to 2.00; *quinquefasciatus*. Riverside Co.; J. N. Belkin; 21 specimens; mean 0.97 ± .08; SD 0.35; range 0.67 to 1.50; *quinquefasciatus*. Orange Co. (fig. 6); Sept. 24, 1953; J. G. Shanafelt; 100 specimens; mean 0.78 ± .03; SD 0.27; range 0.25 to 1.67; 2 intermediates, 98 *quinquefasciatus*. Orange Co.; Sept. 30, 1953; J. G. Shanafelt; 80 specimens; mean 0.79 ± .04; SD 0.35; range 0.30 to 2.50; 1 intermediate, 79 *quinquefasciatus*.



Sardinia

Dr. T. H. G. Aitken provided a number of jounted lots from Sardinia. The coverslips were unsupported on these preparations so they are not strictly comparable with the author's material. Various dates; 104 specimens; mean $-0.11 \pm .00^4$; SD 0.05; range -0.22 to $+0.03$; *pipiens*.

Puerto Rico

Mayaguez. I. Fox; 11 specimens; mean $0.83 \pm .11$; SD 0.36; range 0.57 to 1.50; *quinquefasciatus*.

Liberia

Monrovia. G. J. Burton; 8 specimens; mean $0.90 \pm .14$; SD 0.40; range 0.71 to 1.38; *quinquefasciatus*.

DISCUSSION

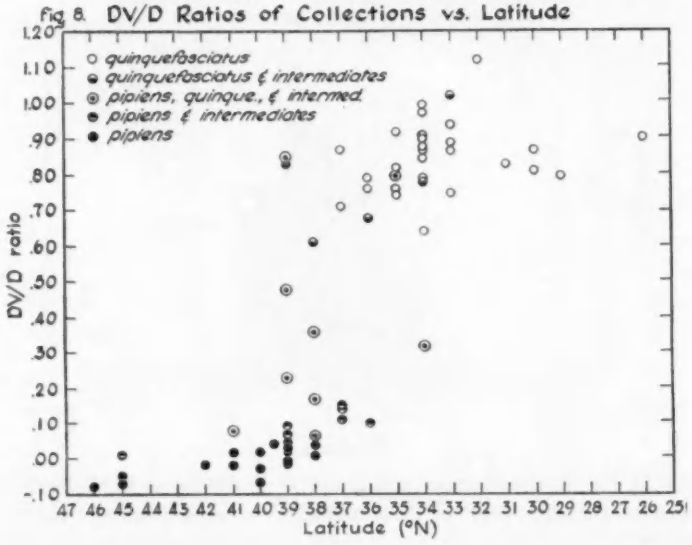
From the foregoing data (Figs. 8 & 9) it can be seen that in general in North America only *pipiens* is found north of about 39°N. and only *quinquefasciatus* south of about 36°N. Between these two latitudes *pipiens*, *quinquefasciatus*, intermediates, and various mixtures of the three are found.

It would appear (Fig. 7) that there is a gradual cline in the *pipiens s. s.* population, smaller values being found in the north and larger ones in the south. This may be due to introgression of *quinquefasciatus* genes from the south. The Norfolk, Virginia collection (Fig. 4) would support this view since it is composed of *pipiens* and intermediates; no *quinquefasciatus* were found in that locality.

There is a steep gradient in the average ratio in the zone of intergradation of the two forms as shown in Fig. 8. Collections from this area

[4] Less than .005.

Fig 8. DV/D Ratios of Collections vs. Latitude



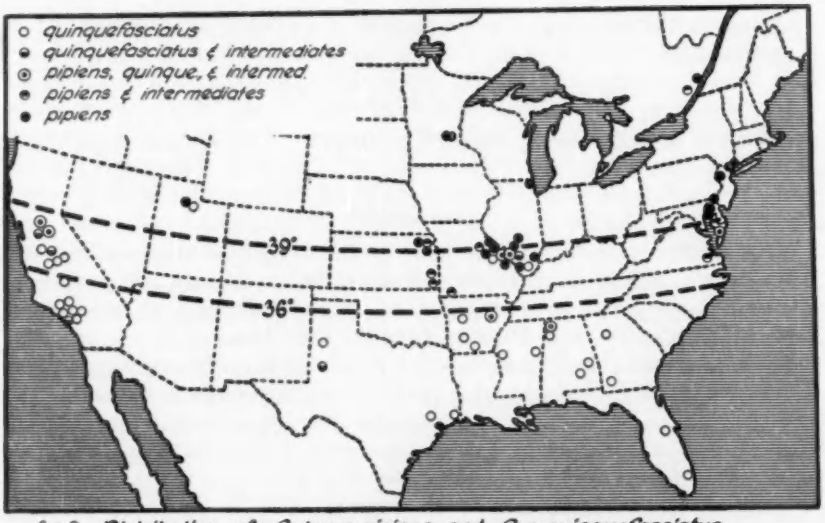


Fig 9. Distribution of *Culex p. pipiens* and *C. p. quinquefasciatus* in America north of Mexico.

are very variable and are composed of mixtures of *pipiens*, *quinquefasciatus*, and intermediates. In most areas (East St. Louis, Fig. 3) the two parental forms are more common than intermediates which suggests that there may be unrecognized isolating mechanisms present. In at least one locality (Sacramento Co., Calif., Fig. 5) the forms are thoroughly intermingled which shows that there is considerable gene flow between the two forms in nature.

The ratio apparently reaches a maximum value in the *quinquefasciatus* population (Fig. 8). No cline can be seen even when collections from equatorial areas are included. In large samples of *quinquefasciatus* a few values may be found which overlap with hybrid values. This is the author's interpretation of the low values in the Orange Co., California collections (Fig. 6) since this locality appears to be so far south of the zone of intergradation (Fig. 9); it probably also accounts for the low values from Lubbock, Texas, and Loma Linda, California.

There is one outstanding *pipiens* collection, that from Bull Shoals, Arkansas, which is in the range of *quinquefasciatus*. This is a mountainous locality which suggests that the cline from *pipiens* to *quinquefasciatus* might be found on the sides of mountains in the *quinquefasciatus* zone.

Systematic position. In some localities (St. Louis, Fig. 3) *pipiens* and *quinquefasciatus* seem to be isolated for the most part even though there appears to be some gene flow between the two forms. In such localities one might be tempted to consider the forms separate species but to the author this makes the species concept very subjective. Since there appears to be some gene flow between the forms in such localities, the author would consider the forms subspecies. In other localities (Sacramento Co., Fig. 5) the forms appear to be thoroughly intermingled. In such localities one would consider the forms to be no more than subspecies at best. In view of the arguments of Wilson and Brown (1953) one should consider the objective reality of the subspecies concept as applied to these forms. At the present time it would appear that each form has a complex of biological characteristics which differentiates it from the other form, although these characteristics have not been studied from a quantitative point of view. These characteristics include the amount of space needed for mating, the ability to overwinter, and preferences in breeding places and hosts. The author therefore favors retention of subspecific status for the two forms although it would be well to study other clines in these forms to learn whether they have steep gradients which parallel the presently discussed one. It should be pointed out that present evidence indicates the inheritance of the DV/D ratio to be multifactorial and not due to one or two genes.

"*Culex molestus*" is a complicating factor in the complex since it appears to have a discontinuous distribution throughout the range of *pipiens*. Since autogeny (the ability to lay eggs without blood) is diagnostic for "*molestus*" this can be considered another biological characteristic of the *pipiens* population; autogeny is unknown in *quinquefasciatus*.

Finally, the author believes that this complex should be studied more intensively in the zone of intergradation between the two forms. There appear to be

isolating mechanisms between *pipiens* and *quinquefasciatus* in most localities which are not recognized at the present time. Hybrid sterility does not seem likely in view of the work of Sundararaman (1949) and Barr and Kartman (1951). Rozeboom and Gilford (1954) were not able to demonstrate "psychological" or behavioral barriers to mating in these forms.

ADDENDUM

Since the above was written the author has received a series of males from the Los Angeles, California area through the courtesy of Dr. J. N. Belkin. These included 55 specimens of which three were *pipiens*, 13 intermediate, and 39 *quinquefasciatus*. The most interesting series was a group of six specimens from Wilmington which had ratios ranging from .03 to .43; three of the six were *pipiens*. From this information it is clear that there is a hybrid swarm on the coast of California near Los Angeles. This information also demonstrates that what the author considered to be normal variants from Orange County, California were actually intermediates. The only other specimens which appeared to be normal variants in this study were those from Lubbock, Texas. Further collecting would probably reveal a hybrid swarm in that area as well.

SUMMARY

The DV/D ratio was measured in about three and a half thousand males of *pipiens* and *quinquefasciatus*, mostly from North America. It was found that the average ratio in a collection of *pipiens* is usually 0.1 or less and in *quinquefasciatus* 0.6 or more. In individual specimens the ratio is usually less than 0.2 in the former and more than 0.4 in the latter. In North America north of 39°N. only *pipiens* is usually found; south of 36°N. only *quinquefasciatus* is generally present. Collections from between these latitudes may contain one or the other form, intermediates, or various mixtures of the three. There seems little doubt but that the forms do hybridize in nature. In some localities (Sacramento, Calif.) intermingling seems to be much freer than in others (East St. Louis, Ill.). At the present time it seems wisest to retain subspecific names for the two forms.

ACKNOWLEDGMENTS

The author would like to express his thanks to the many people who contributed specimens for this study and to Sylvia Barr for the preparation of the illustrations.

REFERENCES

Barr, A. R., and Kartman, L., 1951. Biometrical notes on the hybridization of *Culex pipiens L.* and *C. quinquefasciatus* Say, *J. Parasit.* **37**: 419–420.

Dobrotworsky, N. V., and Drummond, F. H., 1952. The *Culex pipiens* group in south-eastern Australia. II, *Proc. Linn. Soc. New So. Wales* **78**: 131–146.

Knight, K. L., 1953a. Suggestions for the measurement of variation in the *Culex pipiens* complex, *Trans. IXth Int. Cong. Entom.* **2**: 297–300.

———, 1953b. Hybridization experiments with *Culex pipiens* and *C. quinquefasciatus*, *Mosq. News* **13**: 110–115.

———, 1953c. The mosquitoes of the Yemen, *Proc. Ent. Soc. Wash.* **55**: 212–234.

———, AND MALEK, A. A., 1951. A morphological and biological study of *Culex pipiens* in the Cairo area of Egypt, *Soc. Fouad. 1er d'Ent. Bull.* 175–185.

MATTINGLY, P. F., ROZEBOOM, L. E., KNIGHT, K. L., LAVEN, H., DRUMMOND, F. H., CHRISTOPHERS, S. R., AND SHUTE, P. G., 1951. The *Culex pipiens* complex, *Trans. Roy. Ent. Soc. Lond.* **102**: 331–382.

ROZEBOOM, L. E., AND GILFORD, B. N., 1954. Sexual isolation between populations of the *Culex pipiens* complex in North America, *J. Parasit.* **40**: 237–244.

SUNDARARAMAN, S., 1949. Biometrical studies on intergradation in the genitalia of certain populations of *Culex pipiens* and *Culex quinquefasciatus* in the United States, *Amer. J. Hyg.* **50**: 307–314.

WILSON, E. O., AND BROWN, W. L., JR., 1953. The subspecies concept and its taxonomic application, *Syst. Zool.* **2**: 97–111.

CANINE ASCARIASIS AS A POTENTIAL SOURCE OF VISCERAL LARVA MIGRANS[1]

FRANK A. EHRENFORD

Research Department, Pitman-Moore Co., Division Allied Laboratories, New Augusta, Indiana

Recent investigations, summarized by Beaver (1954), have disclosed human visceral larva migrans as a disease of previously unrecognized cause. While this disease is not yet fully described, it is known that children up to four years of age are commonly affected, and adults probably are occasionally involved. Lately, Heiner and Kevy (1956) have added another case involving children, and have diagnosed several others.

Several types of human larva migrans are induced by canine parasitic nematodes, as follows (Beaver, 1954): ophthalmic nematodiasis involving unidentified canine nematodes (hookworms?); cutaneous larva migrans caused by *Ancylostoma braziliense*, *A. canium*, and *Uncinaria stenocephala*; central nervous system nematodiasis; and visceral larva migrans. The visceral type may prove to be the most common, if not indeed the most dangerous in human beings.

Another aspect of migrating nematode larvae is provided in the interesting review by Shoho and Tanaka (1955) regarding domestic animals. These authors speculate on correlations between migrating larvae and the activation of latent virus infections. Evidence is provided by Sprent (1955a, b) to show that ascarid invasion of the central nervous system is not at all unusual under experimental conditions.

Of the two ascarids, *Toxocara canis* and *Toxascaris leonina*, frequently found in dogs and cats respectively, *T. canis* from dogs appears to be the principal etiological agent of human visceral larva migrans. In human beings the disease is produced by ingesting infective eggs of the canine ascarids by either direct or accidental geophagy. Human infection with canine and feline ascarids is a zoonosis (animal to man), and it is etiologically distinct from human infections with *Ascaris lumbricoides* (man to man).

Because of the increased importance of canine ascarids from the human medical standpoint, certain enzootic data become desirable regarding the infection in the reservoirs: viz., incidence, seasonal variation, host sex specificity, and host age preference. Such information would assist in clarifying the scope of the problem.

MATERIALS AND METHODS

Data on qualitative stool examinations of 1,465 naturally infected dogs were recorded for 26 months. Metazoan parasites were diagnosed by means of a centrifugal-flotation procedure employing a sodium dichromate solution of specific gravity 1.36. The dogs came from the Midwest region in the area of the

[1] Presented at the Annual Meeting of American Society of Parasitologists, 1956, Storrs, Connecticut.

Ohio River Drainage (Ehrenford, 1953). Age of the dogs was determined by condition of the teeth (Cadiot and Breton, 1924; Barton, 1939; Sisson, 1953). The data were tested statistically by the chi square method. Data collected during the months of April to August, 1954, were considered biased due to selection of dogs, and were excluded from this study.

RESULTS

Fecal examinations on 1,465 dogs showed an overall percentage incidence of 21.0 for *T. canis*, which value is in agreement with data given by Goss and Rebrassier (1922) as 23.0 in 795 dogs in Ohio, as well as with those of subsequent investigators examining large numbers of animals. It is of interest that the incidence of this infection appears to have remained unchanged during a 33 year period. *T. leonina* was found in 4.1 per cent of dogs in the present study, and *Toxocara cati* was diagnosed in 0.003 per cent (5 animals).

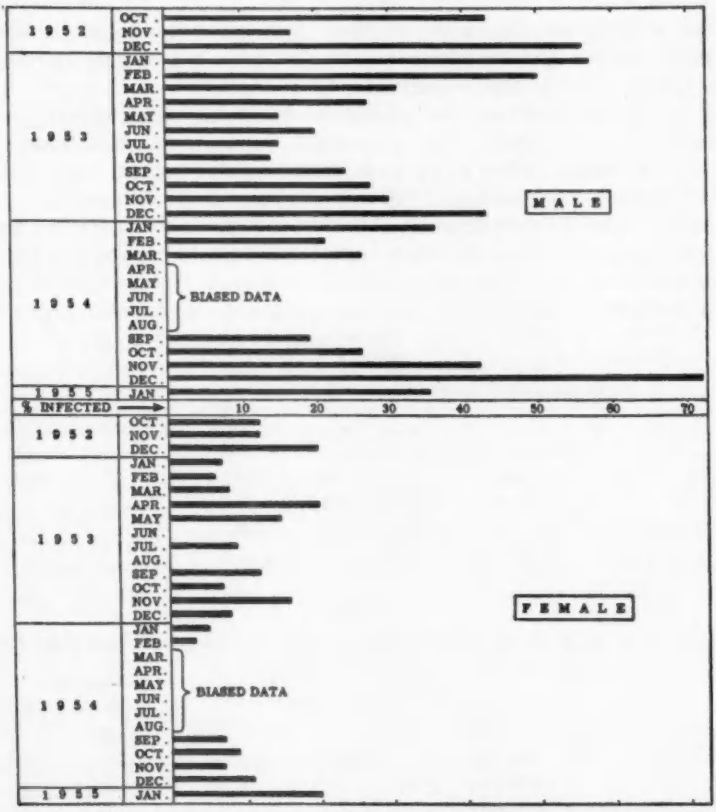


FIG. 1. Monthly per cent of *Toxocara canis* infection in 1,324 dogs (687 males, 637 females) aged 3–36 months.

Analyzing the incidence of *T. canis* with regard to sex of host in 1,324 dogs (687 males, 637 females), it was found that 32.8 per cent of the males and 9.4 per cent of the females were infected (Figure 1). Thus, male infections exceeded female infections by a factor of 3.5. *T. leonina* was found in 3.15 per cent (av.) of both sexes, indicating no differences due to sex of host for this species. Dual infections (*T. canis* and *T. leonina*) were found in only 1.1 per cent (15 animals), of which 80 per cent were in male dogs.

The distribution of *T. canis* infections according to sex and age of host in 1,294 dogs is also of interest (Table 1). Analysis of the values for the males showed no significant differences, indicating that age resistance develops slowly, if at all. In contrast, values for females were significantly different and indicated a marked increasing resistance after the age of 3–6 months. Of special interest were the findings on comparing the values in the similar age groups of males and females; only in female dogs older than 3–6 months was a significant difference found. Younger dogs of both sexes (3–6 mos.) had the highest incidence of infection. The latter observation confirms the conclusion reached by Hinman and Baker (1936). The data further show that after 3–6 months infections in males are significantly higher than those in females.

The use of teeth as a basis for judging the age of dogs after maturity is open to valid criticism because of the considerable variation in sexual maturation; however the criteria suffice to separate immature and mature animals (Table 2). Comparisons of Tables 1 and 2 do not show any major differences.

In males there is a definite seasonal trend for *T. canis* infections, the highest incidence occurring during the winter and the lowest during the summer. The seasonal incidence for female dogs exhibits an irregular fluctuation but there is some suggestion of maximum incidence during the winter months (Figure 1).

TABLE 1

Incidence of T. canis *by sex and age of host in 1,294 dogs*

		3–6 mos.	7–9 mos.	10–12 mos.	13–36 mos.	Av. % infected
Males, total 676	No. dogs	45	76	287	268	36.8
	% infected	44.4	40.7	31.0	31.3	
Females, total 618	No. dogs	55	94	227	242	14.0
	% infected	29.0	13.8	8.4	4.9	

TABLE 2

Incidence of T. canis *by sex and grouping of immature and mature dogs (total 1,294)*

	Immature		Mature	
	Age 3–12 mos.	% infected	Age 13–36 mos.	% infected
Males, total 676	140 Inf. 408 Total	34.2	84 Inf. 268 Total	31.2
Females, total 618	48 Inf. 376 Total	12.7	12 Inf. 242 Total	4.9

DISCUSSION

Ample evidence is available from the work of several authors in the last 33 years to show that the high incidence of *T. canis* infection in puppies originates as a prenatal infection (Yutuc, 1954). This serves to show that anthelmintic therapy must be carried out for several generations in order to control this parasite.

The resistance differential to *T. canis* exhibited by male and female dogs seems best explained as a sex difference, although in the case of females both sex and age may be contributory factors. Evidence that sex of the host is a factor in resistance is reviewed by von Brand (1952). Age resistance to other ascarids has been demonstrated in pigs (Ransom and Foster, 1920) and in chickens (Ackert *et al.*, 1935).

Cats do not appear to harbor *T. canis* to a significant degree in this country as judged from several small surveys which have been conducted during the last 14 years. Their ascarid infection rates may go as high as 62 per cent, but the principal feline ascarids appear to be *T. leonina* and *T. cati*. However, Hitchock (1954) has indicated a 1.0 per cent *T. canis* infection in a small sample, so cats are not entirely free of suspicion as a reservoir.

Sprent (1954) has outlined life-cycle differences in members of the Ascarididae. It appears to be significant that *T. canis* follows a visceral route in its development, while *T. leonina* tends to restrict its invasion of tissue to the gut wall. These observations tend to involve *T. canis* as an agent of human visceral larva migrans and to exclude *T. leonina*.

Especially noteworthy is the strong presumptive evidence provided in the present study to support Beaver's (1954) suggestion that *T. canis* is the main etiological agent in human visceral larva migrans. The larvae of *T. canis* are most frequently found in human tissues (Beaver, 1954) and adult *T. canis* is common only in dogs, especially males. Serological evidence provided by Heiner and Kevy (1956) further strengthens the case in favor of *T. canis* as the principal causative agent.

Visceral larva migrans caused by *T. canis* may be expected to occur wherever canine ascariasis is found. Evidence from canine parasite surveys indicates common occurrence and cosmopolitan distribution of *T. canis*. Human cases may be found to follow a similar trend, since Beaver (1954) indicates that the disease has been diagnosed in 12 states of this country, as well as in England and Puerto Rico.

SUMMARY AND CONCLUSIONS

A high incidence of infection with *T. canis* was found in dogs aged 3–6 months. In mature dogs a marked difference in resistance is found between the sexes. Males exhibit little resistance to *T. canis* as compared to females, whose resistance increases up to the age of 3 years. A pronounced variation in seasonal incidence of *T. canis* was found in male dogs; the incidence was highest during winter and lowest during summer months. Female dogs showed a fairly uniform seasonal incidence. Evidence is provided in this study to show that in the

Midwestern area of the United States *Toxocara canis* is a common parasite of dogs.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of Mrs. Esther Goettling, Librarian, and Mrs. Leona Pratt, Mr. Jack Smith, and Mr. Olin Rhoades of the Biological Plant, Pitman-Moore Co., Zionsville, Indiana.

REFERENCES

ACKERT, J. E., PORTER, D. A., AND BEACH, J. D., 1935. Age resistance of chickens to the nematode, *Ascaridia lineata* (Schneider), *J. Parasitol.* **21:** 205–213.

BARTON, A., 1939. Age determination in dogs, *Vet. Student* **2:** 18–19. (Translation of Cadiot and Breton, 1924).

BEAVER, P. C., 1954. Parasitic diseases of animals and their relation to public health, *Vet. Med.* **49:** 199–202, 205.

CADIOT, P. J., AND BRETON, F., 1924. *Medicine et Chirurgie canines.* Asselin and Houzeau, Paris. (See Barton, 1939).

EHRENFORD, F. A., 1953. The incidence of some common canine intestinal parasites, *J. Parasitol.* **39:** (4 Sect. 2): 34–35.

GOSS, L. W., AND REBRASSIER, R. E., 1922. Demonstration of examination of the feces of the dog for parasitic infestation, *North Amer. Vet.* **3:** 177–178.

HEINER, D. C., AND KEVY, S. V., 1956. Visceral larva migrans: report of the syndrome in three siblings, *New England J. Med.* **254:** 629–636.

HINMAN, E. H., AND BAKER, D. D., 1936. Helminthological survey of 1,315 dogs from New Orleans with special reference to age-resistance, *J. Trop. Med. & Hyg.* **39:** 101–104.

HITCHCOCK, DOROTHY J., 1953. Incidence of gastrointestinal parasites in some Michigan kittens, *North Amer. Vet.* **34:** 428–429.

RANSOM, W. B., AND FOSTER, W. D., 1920. Observations on the life history of *Ascaris lumbricoides*, *U. S. Dept. Agric. Bull.* **817**

SHOHO, C., AND TANAKA, T., 1955 Further observations on cerebrospinal nematodiasis in animals. II. The problem of reinfection by nematodes and clinically silent cases, *British Vet. J.* **111:** 102–111.

SISSON, S., 1953. *The Anatomy of Domestic Animals.* W. B. Saunders Co., Philadelphia, Pennsylvania.

SPRENT, J. F. A., 1954. The life cycles of nematodes in the family Ascaridae Blanchard 1896, *J. Parasitol.* **40:** 608–617.

SPRENT, J. F. A., 1955a. On the invasion of the central nervous system by nematodes. I. The incidence and pathological significance of nematodes in the central nervous system, *Parasitology* **45:** 31–40.

SPRENT, J. F. A., 1955b. On the invasion of the central nervous system by nematodes. II. Invasion of the nervous system in ascariasis, *Parasitology* **45:** 41–55.

VON BRAND, T., 1952. *Chemical Physiology of Endoparasitic Animals.* Academic Press Inc., New York, N. Y.

YUTUC, L. M., 1954. The incidence and prepatent period of *Ancylostoma caninum* and *Toxocara canis* in prenatally infected puppies, *J. Parasitol.* **40:** (Sect. 2): 18–19.

FLY PRODUCTION STUDIES IN URBAN, SUBURBAN, AND RURAL PRIVIES IN SOUTHEASTERN GEORGIA[1]

JOHN W. KILPATRICK AND H. F. SCHOOF

The role of flies in the spread of enteric infection has been established by various workers including Watt and Lindsay (1948) who demonstrated in Hidalgo County, Texas, that the incidence of shigellosis could be reduced by instituting control measures. Although house fly production from privies was not significant in these Texas studies, subsequent investigations in the same area (Kilpatrick and Bogue, 1956) showed that house fly production in dieldrin-treated privies was greater than in untreated privies. Similar results were reported in Phoenix, Arizona, by Schoof and Siverly (1954). These findings implied that the disease vectoring potential of house flies would be increased rather than decreased through the use of chemicals in privies for fly control. To investigate more fully this apparent paradox, a series of studies was established near Savannah, Georgia, to ascertain the effects of chemical treatment upon the species composition of flies produced in privy pits. To obtain a base line for future analyses, the initial studies were devoted to determining the qualitative and quantitative aspects of the fly populations of untreated privies on an annual basis. The results of these initial studies are discussed below.

PROCEDURE

The privies studied were in the vicinity of Savannah, Georgia, and were classed as urban, suburban, or rural depending on their general location. In this area, privies are of the simulated pit type in that at one time or another a pit of 2 to 3 feet in depth was present; however, from continued use these pits have filled to varying degrees. Of those sampled, more than 60 per cent had almost completely filled pits. The shallowness of the pit is a necessity in this coastal area because of the high water table. Privies with pits deeper than 3 feet generally fill with water during the wet season and serve as a source of mosquitoes.

To evaluate accurately the fly emergence from any privy, the pit was prepared as follows:

All holes and openings into the pit were sealed to prevent the entry or exit of flies. In general, this was accomplished by stapling heavy building paper to the privy pit and walls, and by banking earth around the bottom edge of the privy. A spring-hinged cover lined with felt to provide a close fit was installed over the seat opening so that when closed no flies could enter or escape from the privy pit. A hole (6-inch diameter) was cut in the outside wall of the privy approximately 12 inches above ground level. This opening was located where possible on the side of the privy affording the most light. A cone-type trap (8 inches high and 6 inches in diameter) constructed from 36 × 40 mesh copper wire was attached securely over the 6-inch opening. Sponge rubber fastened to

[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia.

FIGURE 1. Privy showing trap in place.

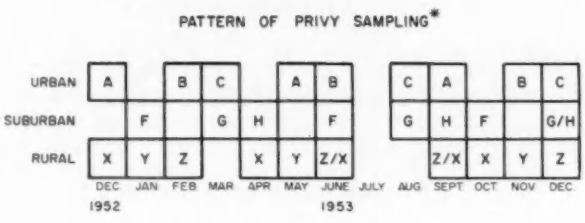


FIGURE 2

the bottom of the trap insured a tight seal. Thus, any flies which emerged inside the privy pit were in darkness except for the light entering through the trap opening. The response of the insects to light induced them to enter the trap. Figure 1 shows a trap in place on a privy typical of this area.

Before trapping of any given privy was started, an effort was made to remove all adult flies in the privy pit. Each privy was then trapped continuously for a period of 3 weeks, after which it was unsealed to allow a resumption of the breeding. Trap catches were removed daily to prevent ant and spider damage.

A total of 100 privies (30 urban, 30 suburban, and 40 rural) was used during the study, 20 privies usually being covered in each 3-week trapping period. Retrapping the same series of privies was done at the intervals indicated in Figure 2. It will be noted that two trapping series were taken in the same environmental area in June, September, and December.

All specimens taken in the 300 privy collections were identified as to species, if possible. Where taxonomic information precluded specific determination, the specimens were determined as to genus or family.

RESULTS

Of the 175,370 specimens of Diptera trapped, 170,401 were identified, this number being classified into 97 species. The latter constituted 97.2 per cent of

the total number of specimens trapped. Identification to genera only represented 0.5 per cent and to family only 2.3 per cent of the total number of specimens trapped. Sixty-five genera and 33 families were represented in the completed tabulation. Of the total specimens trapped, 41,434 were Culicidae, the majority being *Culex quinquefasciatus*.

The voluminous numbers of species and specimens trapped prohibit a quantitative listing of these species. However, a simple tabulation of species, genera, and families follows.

MUSCIDAE

Atherigona orientalis
Coenosia
Dendrophaonia scabra
Fannia canicularis
F. difficilis
F. femoralis
F. howardi
F. leucosticta
F. manicata
F. pusio
F. pusio group
F. scalaris
Fucellia manitima
Hebecnema halterata
Hydrotaea houghii
H. occulta
Hylemya cilicrura
Limnophora arcuata
L. dibilis
L. cilifera
Musca domestica
Muscina assimilis
M. aurantiaca
M. stabulans
Ophyra aenescens
O. leucostoma
Pegomya sp.
Scopeuma furcatum
Stomoxys calcitrans
Synthesiomyia nudiseta

CALLIPHORIDAE

Calliphora livida
C. vicina
Callitroga macellaria
Cynomyopsis cadaverina
Phaenicia caeruleiviridis
P. pallescens
P. sericata
Phormia regina

SARCOPHAGIDAE

Hypopelta scrofa
Sarcophaga assidua
S. bullata
S. cingarus
S. derelicti
S. galeata
S. haemorrhoidalis
S. laakei
S. latisetosa
S. pusiola
S. sarrancenioides
S. ventricosa
Sarcophagula sp.

BIBIONIDAE

BORBORIDAE

Borborus
Leptocera fontinalis
L. sp.
L. venalicia
Sphaerocera sp.

CHLOROPIDAE

Hippelates pusio
H. sp.
Oscinella sp.

CHRYOMYIDAE

Chromya flava

CULICIDAE

A. taeniorhynchus
A. triseriatus
A. vexans
A. sp.
Anopheles crucians
A. quadrimaculatus
Culex erraticus
C. nigripalpis
C. peccator
C. quinquefasciatus
C. salinarius
C. sp.
Culiseta melanura
Mansonia perturbans
Orthopodomyia signifera
Psorophora ferox

DOLICHOPODIDAE

DROSOPHILIDAE

Drosophila busckii
D. melanogaster
D. repleta
D. spp.

EMPIDIDAE

EPHYDRIADAE

Gymnopa sp.

FUNGIVORIDAE

HELEIDAE

HELOMYZIDAE

Amoebaleria
Pseudeleria pectinata
Tephrochlamys rufiventris

ITONIIDAE

LARVAEVORIDAE

LAUXANIIDAE

LEPTIDAE

OMPHALIDAE

Omphrale fenestralis

OTITIDAE

Delphinia picta
Euxesta notata
E. spolita
Rivellia sp.

PHORIDAE

PHYLLOMYZIDAE

Desmometopa m-nigrum
D. sordida
D. sp.
Leptometopa latipes
Milichiella arcuata
M. sp.

PIOPHILIDAE

Piophila casei

PSYCHODIDAE

Brunettia nitida
Phlebotomus sp.
Psychoda alternata
P. severini
P. superba conspicua
P. spp.
Telmatoscopus albipunctatus

SCATOPSIDAE

Scatopse fucipes
S. notata
S. spp.
Swammerdamelia brevicornis

SCIARIDAE

Bradysia spp.

SEPSIDAE

Meroplius stercorarium
Sepsis punctum

STRATIOMYIDAE

Hermetia illucens
Geosargus sp.

SYRPHIDAE

Meromacrus acutus
Tubifera dimidiatus
T. tenax
T. vinetorium

TENDIPEDIDAE

TIPULIDAE

TYLIDAE

Taeniaptera

A tabulation based on family rank is shown in Table 1. These data indicate a wide seasonal variation in the emergence of the different flies. As expected, the total emergence during the winter months (November through February) was considerably less than that during the warmer months. The largest total emergence occurred during September, but of 47,297 specimens trapped, 35,535 were mosquitoes. The emergence of Psychodidae was high during the warmer months, particularly May, September, and October. In general, fly emergence from the urban, suburban, and rural privy areas during any given period was not significantly different.

TABLE 1

Total flies trapped from privies in the Savannah, Georgia, area from December 1952 through December 1953

Type Area	Rural	Urban	Sub-urban	Rural	Urban	Rural	Urban	Sub-urban	Suburban	Rural	Rural	Urban	Sub-urban	Rural	Urban
Month	Dec.	Dec.	Jan.	Jan.	Feb.	Feb.	Mar.	Mar.	April	April	May	May	June	June	June
Muscidae	2479	538	852	1125	798	882	6759	3216	7540	3903	4048	3076	1911	3211	959
Calliphoridae	361	305	178	131	278	260	228	984	644	1076	156	128	16	16	7
Sarcophagidae	2	10	1			1	8	31	36	35	51	44	21	39	24
Bibionidae								2							
Borboridae	33	45	69	39	281	110	434	540	111	224	116	20	9	34	3
Chloropidae			107	13			1	1	1		1	2			
Chryomidae							16	12	58	188	9	14	8	1	3
Culicidae	4	7	1	2	14	2	8	2	6	5	405	15	86	63	974
Dolichopodidae							1	7	0	4	2	3	0	1	
Drosophilidae			1	1	4	20	42	7	2	26	10	4	1	4	5
Empididae										13	4	3			
Ephydridae								13	2		11	1			
Fungivoridae								3	0	2					
Heleidae							1	1	1	5					
Helomyzidae		1			34	48	95	282	1223	406	106	44		2	2
Itoniidae							8	4	18	54	5	5	1	3	
Larvaevoridae							6		0	3					
Lauxaniidae										3					
Leptidae												1			
Orphralidae															
Otitidae									1		1	4	2	1	
Phoridae			119	31	4	9	1	5	1	8	211	17	10	25	16
Phyllomyzidae	1						36	26	360	1615	410	524	185	65	39
Piophilidae												1			
Psychodidae	3	0	88	26	707	406	204	655	125	154	10990	366	676	407	1696
Scatopsidae					76	213	32	32	2	5	154	19	28	155	5
Sciaridae							8	57	12	35	9			1	
Sepsidae							1	10	3	13	68	3	5	29	
Stratiomyidae									38	34	842	151	273	273	153
Syrphidae			11		1		17	78	1	1	350	9	6	9	1
Tendipendidae							2	22	1	1					
Tipulidae								1			2				
Others					8	13	19	6		3	1				
	2883	906	1427	1368	2205	1964	7927	5997	10186	7816	17962	4454	3238	4339	3887

TABLE 1—*Continued*

Type Area	Rural	Urban	Sub-urban	Sub-urban	Rural	Rural	Urban	Sub-urban	Rural	Urban	Rural	Urban	Sub-urban	Sub-urban	Rural
Month	June	Aug.	Aug.	Sept.	Sept.	Sept.	Sept.	Oct.	Oct.	Nov.	Nov.	Dec.	Dec.	Dec.	Dec.
Muscidae	1787	608	302	298	543	239	496	466	426	489	548	368	772	586	388
Calliphoridae	36	28	52	5	6	5	6	35	522	172	411	66	286	302	160
Sarcophagidae	82	7	5	15	22	14	43	16	13	3	2	0	1	4	2
Bibionidae															
Borboridae	4	23	30	41	47	316	203	528	623	1437	182	38	167	88	71
Chloropidae		2	1	1	1			1						0	0
Chryomidae	16	35		1	15	11	13	1	0	1	1	0	0	0	0
Culicidae	648	1073	109	268	112	35535	715	382	386	199	24	95	124	162	8
Dolichopodidae	0	1	5	0	1	1	1	0	0	2	1	0	0		0
Drosophilidae	1	6					1	2	20	324	19	23	4	4	7
Empididae	1	2	2	3	11	11	4	1	2	0	1	0	0	0	0
Ephydridae	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0
Fungivoridae	0	0	0	3	0	5		14	7	0	3	0	0	1	0
Heleidae	0	0	0	0	0	0	3	1	1	3	4	0	0	0	1
Helomyzidae	3	0	0	0	0	1	0	0	0	0	0	1	2	7	3
Itoniidae	3	1	2	4	9	33	21	72	165	4	10	2	2	4	3
Larvaevoridae			1	1											
Lauxaniidae															
Leptidae															
Orphralidae				1											
Otitidae	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phoridae	17	69	3	6	11	14	7	9	6	16	16	0	1	1	0
Phyllomyzidae	357	38	6	21	48	43	51	79	13	61	108	3	1	9	10
Piophilidae														0	0
Psychodidae	761	515	1002	595	250	10946	12565	9253	3743	558	418	5	163	12	3
Scatopsidae	6	10		1	6	2	4	84	5	31	11	3	7	15	4
Sciaridae	3	7	11	11	8		24	19	25	6	11	1	13	9	0
Sepsidae					1	1	6	1	1	1	2	0	0	0	0
Stratiomyidae	318	110	120	118	146	92	154	67	80	1	10	0	0	1	0
Syrphidae	3	1	2	10		6	8	38	17	1	3	2	4	0	0
Tendipendidae					1			1	2	0	3	1	0	0	0
Tipulidae				1	1	22		38	10	2	1	0	7	1	0
Other	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	4048	2536	1653	1405	1239	47297	14325	11108	6068	3313	1791	608	1554	1206	660

Table 2 shows in percentages of total flies recovered the monthly emergence of the 10 major fly species trapped from all privy types. In most instances, these 10 species represented about 50 to 75 per cent of the total number trapped. It is interesting to note that although *H. houghii* was predominant in December (48 per cent of total flies taken), it was completely absent from privies from May through September. *D. scabra* was taken in significant numbers only during March and April. *T. albipunctatus* appears to breed in privies in late summer and early fall. *P. alternata* was particularly prevalent in May.

The predominant species in terms of average monthly emergence per privy is shown in Figure 3. These data include all types of privies. The average fly emergence for the months of March, April, and May were much larger than for the preceding or following months, with *Dendrophaonia scabra* predominant in March and April, and *Psychoda alternata*, the filter fly, in May. Contrary to reports from investigations in other areas as to the absence of *Phaenicia sericata* breeding in privies, 6 per cent (or 871 specimens) of the total fly population emerging from 20 privies in March were this species.

Although representative species from other families emerged throughout the trapping periods in considerable numbers, the predominant species were confined to Muscidae, Psychodidae, and Borboridae. The house fly, *M. domestica*, was not a predominant species during any month. In fact, only during January, June, and September did it approach 5 per cent of the total number of flies emerging. This is in direct contrast with the data from the Rio Grande Valley of Texas where over 95 per cent of the total emergence from treated privies was *M. domestica* (Kilpatrick and Bogue, 1956). Heavy emergence of house flies

TABLE 2

Monthly emergence from privy pits of 10 major fly species, showing percentage of each in relation to total populations trapped

Species	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Aug.	Sept.	Oct.	Nov.	Dec.
Dendrophaonia scabra	1.0	4.0	2.0	40.0	35.0	5.0	13.0	8.0	0.7	0.9	2.3	19.5
Fannia canicularis	20.0	36.0	17.0	4.0	3.0	0.2	0.1	0.0	0.0	*	0.9	*
Fannia pusio group	0.9	6.0	3.0	4.0	12.0	9.0	8.0	3.0	0.3	1.0	4.1	0.1
Hydrotaea houghii	48.0	13.0	10.0	3.0	3.0	*	0.0	0.0	0.0	0.7	5.8	24.5
Musca domestica	2.0	6.0	1.0	0.3	0.3	0.1	5.0	1.0	0.5	0.5	1.6	0.1
Muscina stabulans	4.0	2.0	5.0	6.0	3.0	3.0	0.2	0.3	*	*	*	*
Ophyra leucostoma	0.1	*	2.0	11.0	4.0	13.0	23.0	7.0	0.2	0.3	*	0.1
Phaenicia sericata	0.0	0.2	4.0	6.0	5.0	1.0	0.2	0.8	*	*	0.7	0.1
Psychoda alternata	†	†	†	†	1.0	48.0	1.0	0.2	0.2	2.3	0.3	*
Telmatoscopus albipunctatus	†	†	—	†	*	3.0	21.0	36.0	38.0	73.0	17.0	6.2
Total (10 Species)	76.0	67.2	44.0	75.3	66.3	82.3	71.5	56.3	39.9	78.7	32.7	50.6
Other Spp.	24.0	32.8	66.0	24.7	33.7	17.7	28.5	43.7	60.1	21.3	67.3	49.4

* Less than 0.1 per cent.

† Identified only to psychodidae.

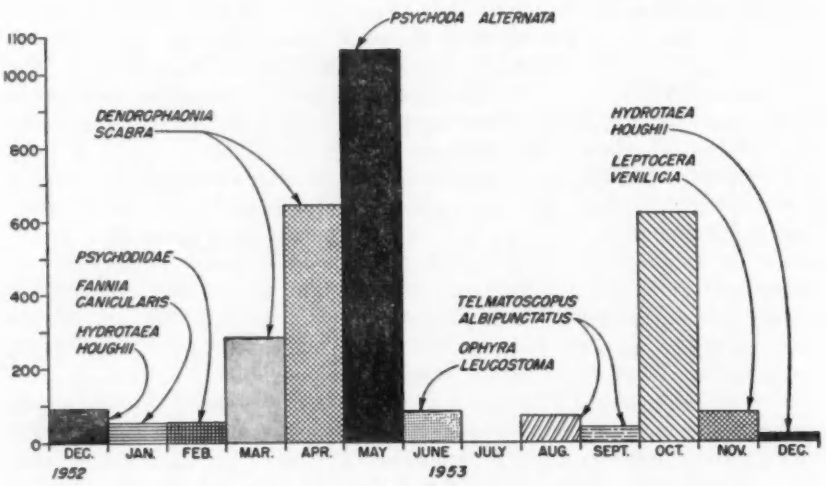


FIGURE 3

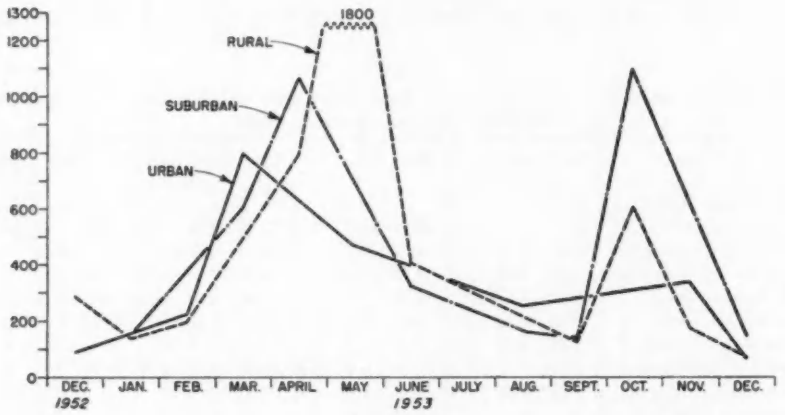


FIGURE 4

from treated privy pits also has been reported from Phoenix, Arizona, and Charleston, West Virginia (Schoof and Siverly, 1954).

The average number of Diptera (exclusive of Culicidae) emerging per privy each month is shown in Figure 4. Spring peak emergence for the urban, suburban, and rural areas occurred in the months of March, April, and May, respectively. The emergence rate declined during the summer months and rose again to a peak in September and October. In this instance, the peak indices for the urban

and rural privies were in September whereas those for the suburban privies occurred in October. In each area, the patterns of increase and decrease in fly populations were similar. The spring emergence peaks from privies contrasted with those of the overall fly populations (largely *M. domestica*) which usually occur during the June-July period in the Savannah, Georgia, area. This variation undoubtedly can be accounted for by the different species involved.

Among the 97 species of Diptera identified, 17 species of Culicidae were found, including representations of the genera *Aedes*, *Culex*, *Mansonia*, *Orthopodomyia*, *Culiseta*, *Psorophora*, and *Anopheles*. *C. quinquefasciatus* was taken in much larger numbers than any other species. Few specimens of *A. quadrimaculatus* were taken.

DISCUSSION

From the results listed above, it is apparent that privies serve as a source of production for numerous species of Diptera. The medical significance of such production must be weighed in relation to the degree and frequency of association and contact with man and his environment. Of the 97 different species collected, only *M. domestica*, *Ophyra* spp., *Drosophila* spp., *C. quinquefasciatus*, and the species of Calliphoridae occur in any significant numbers in homes or food handling establishments; the remainder are rarely encountered by man or are not important as privy breeders.

Although 17 species of mosquitoes were recovered from privies, only one (*C. quinquefasciatus*) can be said to be a privy breeder. Most of the other species were few in number and probably were using the privy as a resting site rather than for breeding purposes.

SUMMARY

Studies were conducted in 100 privies in the Savannah, Georgia, area from December, 1952, to December, 1953, to determine the species composition of the fly populations produced from this source. More than 175,000 Diptera were collected representing 97 species, 65 genera, and 33 families. Patterns of emergence for individual species of Diptera varied seasonably, with *Hydrotaea houghii* most prevalent in December, *Fannia canicularis* in January, Psychodidae in February, *Dendrophaonia scabra* in March and April, *Psychoda alternata* in May, *Ophyra leucostoma* in June, *Telmatoscopus albipunctatus* in August, September, and October, *Leptocera venalicia* in November, and *Hydrotaea houghii* again in December. *Musca domestica* was not an important species at any time, its maximum prevalence being 6 per cent of the total fly emergence during January. No significant variations in the species of flies emerging from the urban, suburban, or rural privies was noted.

REFERENCES

KILPATRICK, JOHN W., AND BOGUE, MALEN D., 1956. Adult fly production from garbage can sites and privy pits in the lower Rio Grande Valley, *Am. J. Trop. Med. & Hyg.* **5:** 331-339.

SCHOOF, H. F., AND SIVERLY, R. E., 1954. Privies as a source of fly production in an urban area, *Am. J. Trop. Med. & Hyg.* **3:** 930-935.

WATT, JAMES, AND LINDSAY, DALE R., 1948. Diarrheal disease control studies. I. Effect of fly control in a high morbidity area, *U. S. Pub. Hlth. Repts.* **63:** 1319-1334.

STUDIES ON ELECTROPHORETIC FRACTIONS OF VIPERA XANTHINA PALESTINAE VENOM[1]

S. GITTER, S. KOCHWA, A. DE VRIES AND M. LEFFKOWITZ

Rogoff Medical Research Institute of the Labour Sick Fund, Beilinson Hospital, Petah Tikvah, Israel

It is well established that the venom of snakes contains a number of components with various pharmacological activities. Until recently the separation of these different components has been difficult and only two crystalline substances have been isolated and reported as chemically pure. Slotta and Fraenkel-Conrat (1938) isolated crotoxin from *Crotalus terrificus* venom, which had neurotoxic and hemolytic but not any clotting activity. Habermann and Neumann (1954, a) showed that the electrophoretic pattern of crotoxin corresponds to a single electrophoretic fraction of whole *Crotalus* venom, but crotoxin has since been further fractionated by Neumann (1955) and by Fraenkel-Conrat and Singer (1956). S. S. De, in 1941, isolated from Colubridae (*Naja naja*) a crystalline hemolysin which is characterized chiefly by its lecithinase (phospholipase A) activity (quoted by Slotta, 1953).

Electrophoretic methods and particularly filter paper electrophoresis with subsequent elution introduced new possibilities for the study of snake venom especially in respect to the distinction and separation of protein fractions (Klobusitzky and König, 1939; Polson, Joubert and Haig, 1946; Grassmann and Hannig, 1954; Habermann, 1954; Grasset and Schwartz, 1955). Species-specific patterns can be demonstrated, although variations in the composition of different batches from one single species exist.

This is a report of the toxicity, of paper electrophoretic pattern and of some toxicologic, pathologic, biochemical and immunological properties of electrophoretic fractions obtained by elution from filter paper of venom of *Vipera xanthina palestinae*, which causes many cases of snake bite every year in Israel (Leffkowitz 1941, 1954a, b, Efrati and Reif, 1953) and is this country's most important venomous snake.

MATERIALS AND METHODS

Venom, obtained by milking adult specimens of *Vipera palestinae*, and dried over $CaCl_2$ at room temperature, appeared as yellow glittering scales. These were preserved, protected from light, at +4°C. A stock physiological saline solution of 100 mg./ml. was prepared and stored at −15°C.

Fractionation of venom by paper electrophoresis. Electrophoresis was carried out with a horizontal type of paper electrophoresis apparatus[2] on Whatman No. 1 filter paper strips of 4 cm. width, in veronal-Na acetate buffer, pH 8.6, ionic

[1] This work was in part supported by a grant from the Executive Committee of the General Federation of Jewish Labour in Israel.

[2] Built in the workshop of the Hebrew University-Hadassah Medical School, Jerusalem, Israel.

strength 0.1. Duration of run was 16 hours, current 4 volt/cm. and staining was done with Amido Black 10B.

For separatory preparation of the various electrophoretic fractions, 0.5 ml. of the stock solution was applied on a 20 cm. wide sheet of Whatman 3 MM filter paper. The run was carried out as above with one change, i.e., increasing the current from 4 to 5 volt/cm. The electrophoretic pattern was determined on narrow strips which were cut out from the middle and from the sides of the sheet. Guided by this pattern the unstained sheet was cut into strips so as to contain separate single fractions. These strips were then eluted by shaking for one hour in two successive portions of 5 ml. physiological saline. Both eluates of each strip were added and stored at −15°C. Eluates of various fractions were concentrated by eluting corresponding strips of three electrophoretic runs with the original 10 ml. of saline eluate.

Toxicity. Intraperitoneal LD_{50} and LD_{100} of the whole venom and of its separate fractions were determined for locally bred Swiss albino mice, using 5 animals per dose. Hemorrhagic and neurotoxic signs were correlated with various dose levels, and the relationship of dosage to early and late death (i.e., 3–7 hours and 12–48 hours post injection) was established.

Determination of hemolytic activity. Direct and indirect hemolysis of human red cells was determined according to the method of Grassmann and Hannig (1954). Direct hemolysis is the lytic effect of venom on red blood cells. Indirect hemolysis is measured by the lytic activity of snake venom on red blood cells in the presence of egg yolk lecithin, which is transformed into lysolecithin by venom phospholipase A. Lysolecithin in turn is the hemolytic factor.

Inhibition of egg yolk coagulation. Venom and various electrophoretic fractions of the venom were mixed with egg yolk solution and maintained at a temperature of 93°C. Coagulation times of the mixtures were compared with normal heat coagulation time of egg yolk solution (Grassmann and Hannig, 1954).

Protective activity of Pasteur antiserum. Specific antiserum against *Vipera palestinae* venom has been prepared by the Institut Pasteur in Paris. Two and 4 LD_{100} doses of whole venom and of separate and combined electrophoretic fractions were incubated for 1 hour at 37°C. with the Pasteur antiserum. Toxicity estimates on mice (5/dose) were made to establish the protective capacity of the antiserum.

Precipitin content of Pasteur antiserum. Precipitin content of the Pasteur antiserum was measured by adding 0.1 ml. of a 1 per cent solution of whole venom or 0.1 ml. of eluate to 0.3 ml. of Pasteur antiserum in increasing saline dilutions. The mixtures were incubated for 2 hours at 37°C. and left overnight at room temperature, when turbidity was estimated.

RESULTS

Toxicity: whole venom. The following LD values for whole venom, administered intraperitoneally to white mice of 15 gm. body weight, were found: LD_{100} = 0.055 mg./mouse (3.6 μg./gm. body weight); LD_{50} = 0.038 mg./mouse (2.5 μg./gm. body weight).

One LD_{100} caused death within 3–7 hours (early death) although, rarely, delayed death after 12–48 hours was observed. With doses greater than LD_{100} death occurred within 1–3 hours. The mice showed increasing weakness, lower body temperature, blanching of ears and tail, impaired movement and finally loss of reactivity. Irregular gasping respiration preceded death from hemorrhagic shock. Doses greater than 2 LD_{100} produced neurotoxic signs—paresis, jerky body movements, convulsions and paralysis of hind legs. Post mortem examination revealed extensive blood clots in the abdominal cavity with hemorrhages in the peritoneum, the pleura, the abdominal muscles and the diaphragm.

Toxicity: fractions. The stained electropherogram of the venom of *Vipera palestinae* (Fig. 1) shows six fractions: one, which moves in cathodic direction (K_1) and 5 anodic fractions (A_1–A_5). Fraction A_4 appeared as a yellow band, visible without staining. Two electropherograms of other venoms are shown in Fig. 1 for comparison, one from the Brazilian *Bothrops jararaca* and one from the S. African *Bitis arietans* belonging to the *Crotalidae* and *Viperidae* respectively.

The sum total of the lethal doses of the different fractions was more than 70 per cent of the total number of lethal doses present in the original quantity of venom applied to the filter paper. The total number of lethal doses in the whole venom was 726 (40 mg. applied to filter paper; one LD_{100} was 0.055 mg.; therefore 40 mg. containing $\frac{40}{0.055} = 726$ LD_{100}). The eluates (10 ml.), according to Table 1, contained respectively 10, 40, 40, 400, 20 and 10 LD_{100} or a total of 520 LD_{100}.

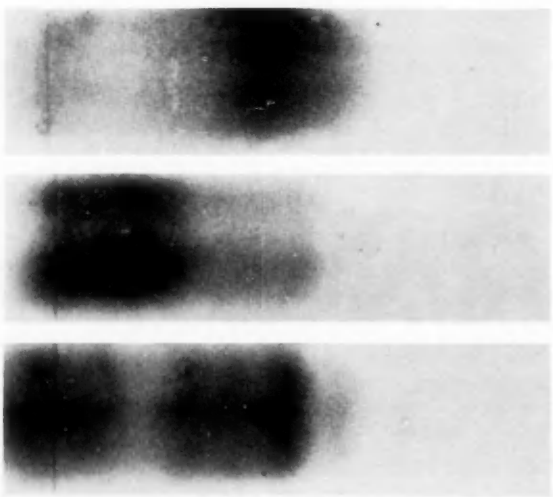


FIG. 1. Electropherograms of snake venoms. Above: *Bothrops jararaca*; middle: *Bitis arietans*; below: *Vipera palestinae*.

TABLE I

Effects of whole Vipera palestinae *venom and of electrophoretic fractions*

	Lethal Dose ml./15 g. Mouse		Effects of 2 LD_{100} i.p.				Coagulation Time of Egg Yolk†	Indirect Hemolysis‡	
	LD_{100}	LD_{50}	Hemorrhagic	Neurotoxic	Early death	Delayed death		Undiluted	Highest dilution producing one plus
							min.		
Whole venom 1% solution	0.055	0.038	+++	−	5/5	0/5	>80	+++	1/8192
Eluate*									
K_1	1	0.75	} −	} +++	} 2/5	} 3/5	2½	+	1/8
A_1	0.25	0.15					2½	+	1/8
A_2	0.25	0.2	++	+	5/5	0/5	>80	+	1/8
A_3	0.025	0.01	+++	−	5/5	0/5	4	++	1/128
A_4	0.5	0.15	} −	} +++	} 0/5	} 5/5	>80	+++	1/8192
A_5	1	−					>80	+++	1/8192
Saline control	—	—	−	−	0/5	0/5	2½	−	—

Effects: +++ = strong, ++ = evident, + = faint, − = no, } = combined concentrated eluates tested.

* 10 ml. saline eluates of each fraction obtained from a run of 50 mg. of whole venom.

† Egg yolk solution 40%, 1 ml.; 1/256 dilutions of whole venom or eluate, 0.2 ml.

‡ Egg yolk, 4% solution, 0.8 ml.; venom or eluate dilution, 0.2 ml.; washed human red cells, 5% suspension, 1.0 ml.

The toxicity of the various fractions was strikingly different (Table 1).

a) *Lethal dose.* The order of toxicity from greatest to least was $A_3 > A_2$, $A_1 > A_4 > K_1$, A_5.

b) *Early and delayed death.* Screening trials showed that fractions K_1 and A_1 had a similar action in respect to early and delayed death. Fractions A_4 and A_5 also showed similarity of toxic and lethal activities. Because of these findings, combined eluates of the fractions K_1–A_1 and of A_4–A_5 were used.

Two LD_{100} of fraction A_2 and of A_3 generally caused early death (within 3–7 hours), 2 LD_{100} of fractions K_1–A_1 generally caused delayed death (after 12–48 hours), while 2 LD_{100} of A_4–A_5 consistently caused delayed death.

c) *Hemorrhagic and neurotoxic actions.* Two LD_{100} of fraction A_2 and of A_3 caused extensive hemorrhage but no signs of neurotoxic disturbance. Conversely 2 LD_{100} of combined concentrated K_1–A_1 and of A_4–A_5 produced marked neurotoxic effects, but no hemorrhage.

The neurotoxic activity of A_4–A_5 however was different from that of K_1–A_1. The injection of the combined fractions A_4–A_5 produced an intense generalized tremor similar to that produced by toxic doses of anti-cholinesterase drugs (e.g., di-isopropyl fluorophosphonate).[3] Slight transient suppression of tremors was achieved by subcutaneous injection of 0.1–0.5 mg. atropine. The K_1–A_1 fractions produced neurotoxic signs similar to those described for whole venom.

[3] After this paper had been submitted for publication, Lee, Chang and Kamijo (1956) demonstrated anticholinesterase action in Formosa snake venom.

Inhibition of coagulation and indirect hemolysis. No direct hemolysis was produced by whole venom or its fractions. Fractions A_4 and A_5 strongly inhibited the coagulation of egg yolk by heat and also caused indirect hemolysis in high dilutions. Fraction A_2 which equally had a strong coagulation inhibitory action, was only slightly active in the indirect hemolysis test.

Effect of Pasteur antiserum. The results of the antiserum titrations on mice are summarized in Table 2 and Figure 2.

Two LD_{100} of whole venom produced death with severe hemorrhages while 4 LD_{100} induced in addition slight neurotoxic signs. To neutralize the activity of increasing doses of whole venom it was necessary to give higher than proportionally increasing doses of antiserum. In fact doses of antiserum which neutralized the lethal and hemorrhagic effects of 4 LD_{100} of whole venom failed to prevent the neurotoxic signs. Large doses of antiserum, much greater than re-

TABLE 2

*Neutralizing effect of Pasteur antiserum**

Venom or Fractions	Dose of Venom LD_{100}/15 g. Mouse	Pasteur Antiserum ml./15 g. Mouse	Toxic Effects						
			Hemorrhagic		Neurotoxic		Death		
			Intensity	Positive/ total	Intensity	Positive/ total	Early	De- layed	Total
Whole	2	0	+++	5/5	−	0/5	4/5	1/5	5/5
	2	0.01	+	2/5	+	2/5	3/5	2/5	5/5
	2	0.025	−	0/5	+	2/5	2/5	3/5	5/5
	2	0.05	−	0/5	+	5/5	0/5	0/5	0/5
	4	0	+++	5/5	±	5/5	5/5	0/5	5/5
	4	0.05	+	5/5	+	2/5	3/5	2/5	5/5
	4	0.1	−	0/5	+	4/5	0/5	4/5	4/5
	4	0.2	−	0/5	±	0/5	0/5	1/5	1/5
	10	4.0	−	0/5	+	5/5	0/5	5/5	5/5
$K_1 + A_1$†	2	0	−	0/5	+++	5/5	2/5	3/5	5/5
	2	0.1	−	0/5	+++	5/5	2/5	3/5	5/5
	2	0.15	−	0/5	+++	5/5	1/5	3/5	4/5
	2	0.2	−	0/5	+++	5/5	2/5	3/5	5/5
A_2	2	0	+++	5/5	−	0/5	5/5	0/5	5/5
	2	0.025	+	5/5	−	0/5	5/5	0/5	5/5
	2	0.05	−	0/5	−	0/5	0/5	1/5	1/5
	2	0.075	−	0/5	−	0/5	0/5	0/5	0/5
	4	0	+++	5/5	±	0/5	5/5	0/5	5/5
	4	0.05	−	0/5	±	5/5	0/5	1/5	1/5
	4	0.1	−	0/5	±	5/5	0/5	1/5	1/5
	4	0.2	−	0/5	+	5/5	0/5	1/5	1/5
$A_4 + A_5$†	2	0	−	0/5	+++	5/5	0/5	5/5	5/5
	2	0.2	−	0/5	+++	5/5	0/5	5/5	5/5

* The venom or fractions were incubated with Pasteur antiserum before intraperitoneal administration.

† Concentrated eluates, combined fractions as described in text.

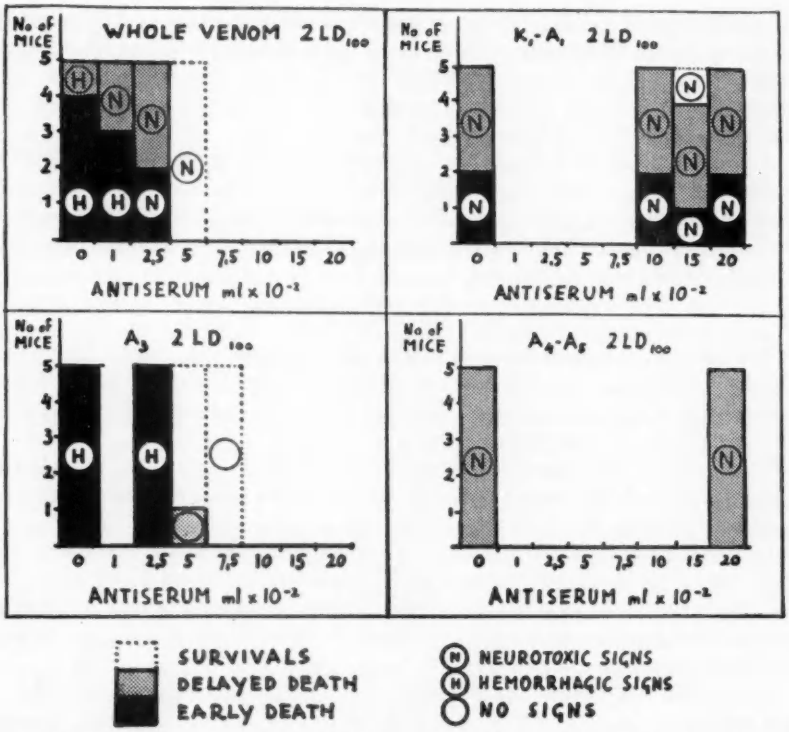


FIG. 2. Differential response of whole venom and of hemorrhagic and neurotoxic components to Pasteur anti serum.

TABLE 3

*Precipitation of whole venom and of fractions by Pasteur antiserum**

Serum Dilutions	Whole Venom 1%	Fractions						Saline
		K_1	A_1	A_2	A_3	A_4	A_5	
1/1	+	±	±	−	±	−	−	−
1/2	++	±	±	−	++	−	−	−
1/4	+	−	−	−	+++	−	−	−
1/8	±	−	−	−	+	−	−	−
1/16	−	−	−	−	±	−	−	−
1/32	−	−	−	−	−	−	−	−
1/64	−	−	−	−	−	−	−	−
Saline	−	−	−	−	−	−	−	−

+++ Strong turbidity, ++ Marked turbidity, + Slight turbidity, ± Traces, − absent.
* Diluted serum 0.3 ml., antigen 0.1 ml.

quired to prevent the lethal effect of 2 LD_{100} of whole venom, did not prevent the neurotoxic and lethal activities of 2 LD_{100} of either combined K_1–A_1 or A_4–A_5. However, the hemorrhagic and the lethal effects of 2 LD_{100} of fraction A_3 were prevented by doses of antiserum of the same order as required for the

neutralization of 2 LD_{100} of whole venom. When the lethal and hemorrhagic effects of higher doses of fraction A_3 (4 LD_{100}) had been prevented by adequate doses of antiserum, it was found that fraction A_3 produced in addition some neurotoxic signs which, however, were slight and transitory and probably due to failure to achieve complete separation.

Precipitation of whole venom and of fractions by the antiserum. The Pasteur antiserum gave a positive precipitin reaction with whole venom. Except for fraction A_3 (hemorrhagic), none of the fractions gave significant turbidity. In fact, this is correlated with the failure of the antiserum to provide protection against the neurotoxic fractions.

DISCUSSION

Venom of *Vipera xanthina palestinae* can be separated electrophoretically into six fractions (K_1, A_1–A_5). These fractions show different activities: lethal, neurotoxic, hemorrhagic, coagulation inhibitory and indirect hemolytic, which however were not uniformly distributed.

Habermann (1954) found maximum toxic activity in one of the five electrophoretic fractions of venom from *Naja nigricollis* and in one fraction of *Naja haje* venom.

Of seven protein fractions which were separated from crude *Crotalus* venom by Slotta (1954) only two were of special interest: a fraction which contained crotoxin with all the principal properties of the crude venom except the proteolytic and coagulating activities and a second fraction which did contain proteolytic and coagulating activity.

Grassmann and Hannig (1954) showed that separate fractions occurred in the venom of the black cobra, one with lecithinase A and the other with egg yolk coagulation inhibitory action. Habermann and Neumann (1954 a, b), investigating *Naja nigricollis* venom, ascribed both these activities to one enzyme—phospholipase A.

Our results show that in the venom of *Vipera palestinae* the early lethal activity (death within 3–7 hours) was mainly concentrated in fraction A_2 which contained 400 out of the total of 525 LD_{100} which were recovered by elution. Hemorrhagic activity was also concentrated mainly in fraction A_3. Neurotoxic activity was concentrated in fractions K_1–A_1 and A_4–A_5, which never produced hemorrhage. Fractions A_4–A_5 consistently caused delayed death (12–48 hours), while K_1–A_1 produced either early or delayed death. The indirect hemolytic activity is concentrated in fractions A_4 and A_5; the slight indirect hemolytic activity of fraction A_2 might be due to tailing during the paper electrophoresis.

No correlation was found between coagulation inhibition and indirect hemolysis. The coagulation inhibitory activity of fraction A_2 is not due to tailing since fraction A_3 does not have such activity. No correlation could be established between the in vivo activity of the individual fractions (lethal, hemorrhagic, neurotoxic) and the in vitro activities investigated (enzymatic).

Whole venom incubated with Pasteur antiserum lost its hemorrhagic and

early death-producing properties but retained neurotoxic activity. The amount of antiserum required to protect the animals from hemorrhage and early death caused by whole venom is proportional to the number of lethal doses injected. The same amount of antiserum per one LD_{100} was found to protect the animals against the hemorrhagic effect and early death provoked by the administration of fraction A_3. It is pertinent that the antiserum contains a high concentration of precipitin mainly directed against the hemorrhagic fraction A_3. However, with high doses of whole venom, relatively large amounts of antiserum did not prevent the appearance of neurotoxic signs and delayed death. When a dose of 10 LD_{100} was administered, 0.4 ml. of antiserum per one LD_{100} did not suffice to save the animals in spite of the fact that no hemorrhages appeared. It is therefore evident that the Pasteur antiserum tested possesses pronounced antihemorrhagic, but little antineurotoxic activity.

The presence of neurotoxic properties in *Vipera xanthina palestinae* venom, in addition to the well known hemorrhagic properties, has been established. These neurotoxic activities of the *Vipera palestinae* venom became manifest after the elimination or suppression of the hemorrhagic picture. This was achieved either by injecting the fractions K_1–A_1, A_4–A_5 which had neurotoxic but were free of hemorrhagic activity, or by injecting whole venom preincubated with adequate amounts of Pasteur antiserum in order to counteract the hemorrhagic activity. High doses of untreated whole venom in many cases showed neurotoxic phenomena which were revealed in spite of the dominant hemorrhagic picture.

The clinical picture of the *Vipera palestinae* snake bite syndrome: swelling at the site of the bite, local and generalized hemorrhage, abnormal hematological findings and anaphylactoid shock, has been described (Efrati and Reif, 1953). Up to now the importance or even the existence of active neurotoxic components in the venom of *Vipera palestinae* have been disregarded. However certain clinical phenomena: persistent vasomotor disturbances (Leffkowitz, 1941), restlessness, urine retention, disturbances in swallowing, and peripheral neuritis (Efrati and Reif, 1953) occurring after *Vipera palestinae* bite in human beings, may well be related to the neurotoxic components of the venom reported in this study. As the neurotoxic fractions K_1–A_1 and A_4–A_5 produced delayed shock and death in mice, the occurrence of delayed shock and death in human snakebite victims after initial improvement and recovery from hemorrhagic lesions (Stern, 1954; Leffkowitz, 1954 a) may also be related to the neurotoxic components of the venom.

It appears that the concentration of the neurotoxic components in the *Vipera palestinae* venom may be of decisive importance for the prognosis of this snake bite. The hemorrhagic components can be neutralized efficiently by the available specific antihemorrhagic Pasteur serum. However no adequate therapy exists at the moment for the neurotoxic aspect of the *Vipera palestinae* bite syndrome. Investigations of the antigenic properties of the neurotoxic venom fractions obtained by paper electrophoretic separation and of the therapeutic effect of a specific antineurotoxic serum are being carried out.

SUMMARY

Six different fractions from the venom of *Vipera xanthina palestinae* were separated out electrophoretically. Eluates of these fractions were tested in mice for lethal, hemorrhagic, neurotoxic and enzymatic activities.

About 80 per cent of the lethal activity of whole venom was concentrated in the A_3 fraction which was dominantly hemorrhagic. Combined eluates of fractions K_1–A_1 and of A_4–A_6 were exclusively neurotoxic. Indirect hemolytic activity was strongest in fractions A_4 and A_5, while egg yolk-inhibitory activity was preponderant in fractions A_2, A_4 and A_5.

Specific *Vipera palestinae* antiserum (Pasteur Institute) neutralized the hemorrhagic action of whole venom and of fraction A_3 but it had very little activity against the neurotoxic components of the venom. The bearing of these findings on the pathogenesis of *Vipera palestinae* snake bite syndrome in human beings is obvious.

ACKNOWLEDGEMENTS

Thanks are due to Dr. H. Mendelssohn and to Mr. A. Tur-Kochba, the Institute of Natural Science, Tel Aviv, and to Dr. J. H. Mason, South African Institute for Medical Research, Johannesburg, for a generous supply of dried venom of *Vipera palestinae* and *Bitis arietans*; to Dr. P. Yekutiel, Ministry of Health, Jerusalem, Israel, for providing the Pasteur antiserum, and to Mrs. C. Aschheim-Poliakoff for technical aid.

REFERENCES

EFRATI, P., AND REIF, L., 1953. Clinical and pathological observations on sixty five cases of viper bite in Israel, *Am. J. Trop. Med. & Hyg.* **2:** 1085–1108.

FRAENKEL-CONRAT, H. AND SINGER, B., 1956. Fractionation and Composition of Crotoxin, *Archives of Biochemistry and Biophysics*, **60:** 64–73.

GRASSET, E. AND SCHWARTZ, D. E., 1955. Fractionnement par électrophorèse sur papier du venin de *Vipera russelii*. Propriétés et dosages des facteurs coagulant et anticoagulant de ce venin, *Annales de l'Institut Pasteur*, **88:** 271–281.

GRASSMANN, W., AND HANNIG, K., 1954. Elektrophoretische Untersuchungen an Schlangen- und Insekten Toxinen, *Hoppe-Seyler's Ztschr. f. Physiolog. Chemie* **296:** 30–44.

HABERMANN, E., 1954. Zur pharmakologischen Charakterisierung elektrophoretischer Fraktionen der Gifte von *Naja nigricollis* und *Naja haje*, *Hoppe-Seyler's Ztschr. f. Physiolog. Chemie*, **297:** 104–107.

HABERMANN, E., AND NEUMANN, W., 1954a. Beiträge zur Charakterisierung der wirksamen Komponenten von Schlangengiften, *Arch. exper. Path. u. Pharmakol.* **223:** 388–398.

HABERMANN, E., AND NEUMANN, W., 1954b. Die Hemmung der Hitzecoagulation von Eigelb durch Bienengift—ein Phospholipase Effekt, *Hoppe-Seyler's Ztschr. f. Physiolog. Chemie* **297:** 179–193.

KLOBUSITZKY, D. V., AND KÖNIG, P., 1939. Biochemische Studien über die Gifte der Schlangengattung *Bothrops*, *Naunyn-Schmiedebergs Arch. exper. Path.* **192:** 271–276.

LEE, C. Y., CHANG, C. C. AND KAMIJO, K., 1956. Cholinesterase inactivation by snake venoms. *Biochem. J.*, **62:** 582–588.

LEFFKOWITZ, M., 1941. Snake bites in Palestine, *Harefuah* **21:** 117–120.

LEFFKOWITZ, M., 1954a. Snake bites in Israel during 1953, *Harefuah* **46:** 82–83.

LEFFKOWITZ, M., 1954b. How a snake strikes, *Dapim Refuiim* **13:** 365.

NEUMANN, W., 1955. Chromatographische Zerlegung von Crotoxin in zwei verschiedene Wirkstoffe, *Naturwissensch.* **42:** 370.

POLSON, A., JOUBERT, F. G., AND HAIG, D., 1946. An electrophoretic examination of cobra venoms, *Biochem. J.* **40:** 265–269.

SLOTTA, K., 1953. Zur Chemie der Schlangengifte, *Experientia* **9:** 81–88.

SLOTTA, K., 1954. Further experiments with crotoxin, *Abstracts International Conf. on Animal Venoms, A.A.A.S.* p. 21

SLOTTA, K., AND FRAENKEL-CONRAT, H. L., 1938. Schlangengifte, III Mitteil.: Reinigung und Krystallisation der Klapper-schlangen-Gifte, *Berichte dtsch. chem. Ges.* **71:** 1076–1081.

STERN, J., 1954. Fatal snake bites, a report of six cases, *Harefuah* **46:** 76–78.

BOOKS RECEIVED

African Izodoidia. I. Ticks of the Sudan, by Harry Hoogstraal. 1101 pages. Wash., D. C., U. S. Govt. Printing Office, 1956.

Home Health Emergencies, ed. by The Equitable Life Assurance Society of the United States. 256 pages. New York, The Life Assurance Society of the United States, 1956.

Lehrbuch der Tropenkrankheiten, ed. by Ernst Georg Nauck. 432 pages. New York, Intercontinental Medical Book Corp., $15.25.

The Happy Life of a Doctor, by Roger I. Lee. 278 pages. Boston, Little, Brown & Company, 1956. $4.00.

Historia da Lepra nó Brasil, Vol. III. Periodo Republicano (1890–1952), by de Souza-Araujo. 715 pages. Rio de Janeiro, Departamento de Impresna Nacional de Brasil, 1956.

BOOK REVIEWS

Industry and Tropical Health II. Proceedings of the Second Conference of the Industrial Council for Tropical Health. Sponsored by The Harvard School of Public Health. April 20, 1954 in New York City, April 21, 22, 1954 in Boston, Mass. Published by The Harvard School of Public Health for The Industrial Council for Tropical Health. 266 pages. $10 (Professional Rate $5).

In format, this volume is comparable to the first proceedings (reviewed in this journal Sept., 1952). The Subject matter, however, is organized more along the lines of specific diseases and disease vectors than was the case with the earlier book.

In order to include and appeal to some executives of tropical ventures, the first day's discussions were held in New York and centered around economic and social aspects of health services in the tropics. Dr. Thomas Weller also presented "Recent Developments in Poliomyelitis" at that time. When the Conference subsequently convened in Boston, most of the presentations of recent information on a disease or method of control were made by experts on the subject with discussion by resident physicians in tropical areas. This pattern is examplified not only by Weller's topic but also by "Trachoma" (Phillips Thygeson); "Arthropod-Borne Viruses in the Tropics" (Harold Johnson); and "Human Performance in Excessive Heat" (C. P. Yaglou). This procedure leads to fairly comprehensive reporting on several topics of medical significance with discussion tying the material to the specific tropical interests of the Conference members. On the whole, the material of the Proceedings has a more universal appeal than its title might suggest.

Besides the subjects mentioned above, one might call attention to "Therapy and Chemoprophylaxis of Malaria," by D. G. Davey; "Amebiasis: New Development in Diagnosis and Treatment," by T. B. McGath; and "Current Trends in the Treatment of Tuberculosis," by A. S. Pope. The book includes a total of 37 presentations with the invited discussions.

B. D. TEBBENS

Practical Medical Mycology, Publication Number 248 American Lecture Series, a monograph in the Bannerstone Division of American Lectures in Internal Medicine, by EDMUND L. KEENEY, A.B., M.D., formerly in charge of Mycology Laboratory, The Johns Hopkins Hospital. Edited by R. L. Pullen, M.D., Dean, University of Missouri School of Medicine, Columbia, Missouri. 145 pages. Springfield, Illinois: Chas. C. Thomas Co. 1955. Price $4.50.

The introduction to this small moderately priced book does not declare the author's intentions for what particular members of the medical profession it was written. Following a reading of the text it is evident that the general practitioner, medical student and public health worker would find it of much value. In all probability the practicing mycologist or the laboratory worker concerned with the isolation of fungi from clinical materials would rely on more extensive books in the field. So often our present-day bacteriologic texts treat

mycology in somewhat of a cursory manner. In a similar vein courses in bacteriology or microbiology relegate the consideration of the mycoses to a minor position with regard to the subject matter presented. This book would indeed effectively make up for shortcomings of some of our texts and if adopted for student use provide a stimulus to seek information about the mycotic infections and their causative agents.

Of the sixteen chapters included twelve are devoted to a consideration of the deep or systemic mycoses. The dermatophytes and their associated diseases are considered within the limits of one chapter. In view of the author's interest in allergy a section is included wherein the role of the spores of saprophytic fungi and their relation to allergic phenomena is presented. The book concludes with sections on mushroom poisoning (mycetismus) and ergotism.

Although the text is of modest size Dr. Keeney very effectively gives us the pertinent information relative to all of the diseases which are now accepted as being caused by fungi. Each disease is treated with respect to its historical background, geographic distribution, its age, sex, race and occupational incidence and its source or sources of infection. There is a discussion of the symptomatology of the disease, pertinent physical and laboratory findings, descriptions of the important roentgenogram findings, mycology, immunology, differential diagnosis, prognosis and treatment.

The textual material for each disease is accompanied by a full page diagrammatic illustration depicting the causative organism, the geographic distribution of the disease on a world-wide basis, sources of infection, clinical picture (primary symptoms, and foci of infection), types of clinical specimens for examination, findings obtained by direct microscopic examination, structural features of the fungus or fungi involved as grown on appropriate media and the differential diagnosis. In those instances where the type of fever, serologic findings and skin test reactions would be helpful in establishing a diagnosis they are also indicated in the illustration. These illustrations contain a mass of information which is clearly delineated and should cause no confusion to the reader.

There is little formal taxonomy in the text, in fact only one "key" giving the characters of the family and genera of the order *Actinomycetales* is included. In those instances where difficulty or confusion has been encountered with the respect to the nomenclature of the specific fungus or of the associated disease the historical background is presented for the acceptance of the names now in general use. In the past the taxonomy of the fungi associated with disease in man was chaotic to say the least and it is no wonder the clinician and the laboratory worker met the problem with despair and defeat. The American mycologists probably more than any other group have brought order out of taxonomic chaos. That they have been successful is clearly reflected in Dr. Keeney's text. His approach to the problem of nomenclature can well be appreciated when he notes "Provincialism has unfortunately crept into the nomenclature employed for describing the various clinical expressions of coccidioidomycosis" and "Though the endemic areas in North America now encompass most of the southwestern United States and northern Mexico, the regional names of the California endemic sites are still fondly, but lamentably, used in describing the various clinical entities of this infection".

The reviewer like the author is "tempted to remain cautious in accepting the old dogma that coccidioidomycosis is never contagious" and disagrees with the common classroom belief that the "spherules" are not contagious. Caution should likewise be exercised with regard to the interpretation of the role played by *Candida albicans* in such conditions as stomatitis, glossitis, black hairy tongue, perlèche, diarrhea, anal and vulvul pruritis and genitocrural involvements where these conditions have appeared following the use of wide-spectrum antibiotics. It is Dr. Keeney's contention "that, although *C. albicans* is found in abundance in the mouths and gastrointestinal tracts of persons receiving wide-spectrum antibiotics and is isolated in the presence of some unfavorable side-reactions resulting from these drugs, the diagnosis of moniliasis in a great majority of these individuals is unfounded".

There is a helpful list of references at the end of each chapter. Both a subject index and name index are provided. Dr. Keeney has drawn upon his extensive laboratory and clinical

experience to produce this most informative text and it should be read with pleasure and profit by anyone who is interested in obtaining a clear conception of the present status of medical mycology.

HERBERT G. JOHNSTONE

Composting: Sanitary Disposal and Reclamation of Organic Wastes, by HAROLD B. GOTAAS, Professor of Sanitary Engineering, University of California, Berkeley, California. Geneva, WHO, distributed in the U. S. A. by Columbia University Press, 2960 Broadway, New York 27, N. Y. 1956. Price £1 5 s., $5.00, Sw. fr. 15.

Much has been written about the fertilizing value of organic wastes, but with too little attention to the question of the danger to public health inherent in the disposal of wastes onto the land. This book emphasizes the principles underlying the safe disposal of community wastes, relating them at the same time to the factors governing the recovery of nutrients from the soil. The public health and economic aspects of composting are reviewed in their historical development from the earliest simple technics to the most modern mechanized processes. The methods of composting recommended for large towns, villages and individual farms are described and all concerned with the problems of disposal and reclamation—small farmer and municipal authority alike—will find this book an instructive and practical guide.

L. W. HACKETT

Cryptococcosis, Torulosis or European Blastomycosis, by M. L. LITTMAN, M.D., Ph.D., Department of Microbiology, The Mount Sinai Hospital, New York, and LORENZ E. ZIMMERMAN, M.D., Central Laboratory, Veterans Administration, Armed Forces Institute of Pathology, Washington, D. C. 205 pp. illustrated with 4 color plates. Grune & Stratton, Inc. New York, 1956. $8.50.

In this authoritative work on cryptococcosis, (the first in a proposed series of monographs on individual mycoses), the subject is presented mainly from the view point of the physician and the medical pathologist, but the more mycological interest is well sustained in a large section on Laboratory Studies. The chapters dealing with clinical features and pathology are profusely illustrated with admirable photographs and photomicrographs and some superb color plates, constituting a veritable atlas of cryptococcosis without entailing any sacrifice of the text.

In the matter of treatment, it must be admitted that chemotherapy has proved very disappointing in cryptococcosis, and surgical measures can be applied only to a very limited extent. It is appropriate, therefore, that the problem of treatment has been discussed at considerable length, and the prospect of success with some of the newer antifungal compounds fully explored in the light of experimental results. The disease in the lower animals may have little epidemiological significance, but its economic importance, particularly in relation to the dairying industry, cannot be gainsaid; as the authors have shown clearly in the chapter on cryptococcosis in animals. An addendum and a useful appendix on special culture media and staining methods complete the work.

This book, concisely written in an attractive and interesting style, reflects the special experience of its authors, and the appended bibliography of 504 titles bears witness to the extensive research which has helped to make it a most complete and satisfying work and a valuable addition to the literature of medical mycology.

J. T. DUNCAN

Diseases of the Chest, by H. CORWIN HINSHAW, M.D., Ph.D., Clinical Professor of Medicine, Stanford University School of Medicine, and L. HENRY GARLAND, M.B. B.Ch., Clinical Professor of Radiology, Stanford University School of Medicine. 634 pp., illustrated. Philadelphia: W. B. Saunders Company, 1956. Price $15.00.

This unusual book entitled "Diseases of the Chest" is a very comprehensive compendium of the diagnosis, treatment and prevention of pulmonary disease. It is an up-to-date,

thorough and accurate portrayal of pulmonary lesions by an able roentgenologist working with a staunch clinician and a highly scientific investigator in thoracic disease. The text is carefully planned, the selection of cases for x-ray demonstration is excellent and only a larger size of x-ray reproduction would have contributed to their value.

The introduction, containing all kinds of diagnostic procedures from the patient's history of complaints to bacteriological concepts, including bronchoscopy, fluoroscopy, measurement of pulmonary function and radiological techniques is a stimulating background for the wide variety of clinical x-ray analyses that are presented. Under the subject of fluoroscopy one would like to have found more factual data on the simple clinical observations concerning chest and diaphragmatic motion as it pertains to the movement of alveolar air in the normal and abnormal chest, a concept that is important in the differentiation of obstructive emphysema, restrictive fibrosis, air trapping and cardiovascular changes associated with pulmonary diseases.

The chapters on tuberculosis may be regarded as the best sections in the book, as would be expected from Dr. Hinshaw's pioneer investigations with Dr. Feldman into the chemotherapy of tuberculosis from the very beginnings of streptomycin therapy. His chapters on medical treatment of tuberculosis, chemotherapy, surgical collapse of the lungs and excision surgery are excellent and should serve as practical background and reference data for the student and the practitioner of medicine and chest diseases, especially in the home care of tuberculosis.

Suppurative disease in its varied forms has been thoroughly handled. Divergence of opinion from the authors' recommendations on medical or surgical therapy may well be based on the continental division of an East Coast reviewer and West Coast authors. Such subjects as superinfection from too broad a spectrum of chemotherapy as well as agammaglobulinemia in recurrent pulmonary suppuration may well be local Boston phenomena and subject to debate. The distinction between the original benignity of so-called Loeffler's Syndrome and the more serious collagen disease is well taken. Sarcoid and the granulomatous pulmonary problems are well handled except that only Californians would recommend sunshine for Boeck's Sarcoid.

The chapters on Fungus Diseases would appear to be brushed off a touch too lightly and briefly, but none the less, if they are read carefully, they are found to contain most of the essential data with extraordinary accuracy regarding the diagnosis of these mycotic infections and their confusion with tuberculosis. Even *Nocardia Asteriodes*, that kaleidoscopic and elusive fungus that tests our diagnostic acumen at every turn, could take a whole chapter for itself. But what the authors say of *Nocardia* should be measured by its weight in gold, not printer's ink. The book is conspicuous for the few omissions in pulmonary disease. The reviewer's interest in pulmonary alveolar microlithiasis makes him wish it had been included as a disease that is not as rare as it seems; but perhaps this entity has too recently reached the Bay Area.

This book is really a masterpiece of work on Pulmonary Disease and is recommended for the internist, the general practitioner, the radiologist, the thoracic physician and surgeon and the teacher and the clinical investigators in pulmonary diseases. If it is studied with care, there will be better diagnosis and treatment of pulmonary disease, better and more highly selective pulmonary surgery, and an awakened intellectual curiosity into the whole field of thoracic disease.

THEODORE L. BADGER

Annual Epidemiological and Vital Statistics, 1953. (Bilingual edition, French and English), 571 pages, World Health Organization: Geneva, Switzerland and New York, distributed by Columbia University Press. 1956. £2 10s., $10.00, Sw. fr. 30.-

This volume is the sixth such collection published by the World Health Organization. The previous five volumes cover the years 1939 to 1952 inclusive, and the WHO series is itself a continuation of the Annual Epidemiological Reports published by the Health Organization of the League of Nations, covering the years 1922 to 1938.

The present volume consists of an Introduction, which discusses the reliability and comparability of the data; Part I, Vital Statistics and Causes of Death; and Part II, Cases of and Deaths from Notifiable Diseases.

Part I contains (a) five tables of demographic data which are essentially a supplement to the U. N. Demographic Yearbook for 1953, (b) tabulations of deaths by cause, age and sex for 35 countries, and (c) several special tables dealing with deaths among children, deaths from tuberculosis and malignant neoplasms, and death rates from selected causes. The classification of causes is according to the abridged "B" list of the 1948 revision of the International List. Some data for 1952 are given, filling gaps in the preceding volume.

Part II covers 34 notifiable diseases, and includes a table of notifiable diseases by country for 1953. (This table is mistakenly entitled "Notifiable Diseases for Various Countries in 1952" in the Table of Contents.)

The major differences between this volume and its predecessor consist of a few changes in the countries covered, plus a new tabulation of death rates by age, sex and country for "diseases of the cardiovascular system", causes B22, B24–29, A85 and A86. The increasing interest in cardiovascular disease makes this tabulation a valuable one, if it is used with caution.

In summary, this publication contains the most recent and accurate data of this type available for 35 countries with a total population of over 500,000,000.

W. GAFFEY

CORRIGENDUM

In the paper by Chaffee, Fife and Kent on "Diagnosis of *Trypanosoma cruzi* infection by complement fixation," in the September, 1956, issue of the Journal, the numbers of the tables on pages 766 and 767 were transposed; Table 1 should be numbered Table 2 and vice versa.

NOTICE TO CONTRIBUTORS

The Journal welcomes papers by members or nonmembers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

Papers should be written in *English.*

Manuscripts should be typewritten using *double or triple spacing* on *one side* only of 8½ x 11 inch paper, if available, and submitted in duplicate. Double spacing should be used throughout the manuscript *including the list of references.*

Tables should be typed on separate sheets and be serially numbered.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

Snyder, T., and Meleney, H., 1941. The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21**: 63–73.

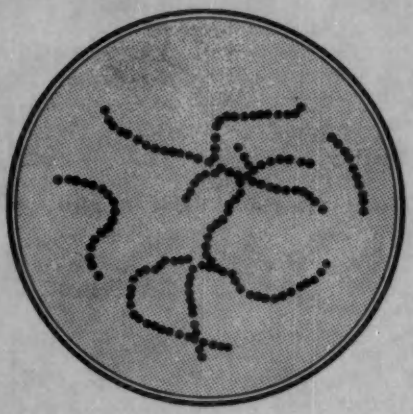
Citation in the text should be by author and date.

Proprietary names of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U.S. Pharmacopoeia, New and Nonofficial Remedies of the A.M.A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

All papers should end with a brief summary.

Estimating costs of reprints. Where the cost of reprints must be known well in advance of publication in order to secure prior approval for their purchase, the Editor will be glad to furnish an estimate of the number of pages which the article will occupy in the Journal.

DIFCO



A S T O

Difco reagents for the diagnosis of

Group A Streptococcal Infections

- RHEUMATIC FEVER
- GLOMERULONEPHRITIS

ANTISTREPTOLYSIN *O* TITERS (ASTO) and their relation to pathological conditions in Group A streptococcal infections have established the importance of this determination as a routine clinical test.

Bacto-Streptolysin O Reagent- a dehydrated, standardized and stable reagent requiring only rehydration with distilled water. Antistreptolysin *O* titers have been impractical for routine diagnosis because of the difficulties in preparing the reagent. Bacto-Streptolysin *O* Reagent is a standardized preparation permitting the routine performance of this diagnostic test in all clinical laboratories.

Bacto-ASTO Standard- an antiserum titred in Todd units for use as a control in the determination of antistreptolysin *O* titers.

Descriptive literature sent upon request

DIFCO LABORATORIES

DETROIT 1, MICHIGAN